

# Bulletin of the Agricultural Chemical Society of Japan.

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## *The Agricultural Chemical Society of Japan.*

President : Teizo TAKAHASHI.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Editor : Teizo TAKAHASHI.

Associate Editors : Kakuji GOTŌ and Yoshihiko MATSUYAMA.

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## Über zwei Neue Glykoside der Tabakblätter. (I).

Über die Chemischen Bestandteile des Tabaks.—II. Mitteilung.

Von

KAZUO YAMAFUJI.

(Aus dem Agr.-chem. Laboratorium der Univ. Kyushu.).

(Eingegangen am 10. Nov. 1931).

### (A) Tabacinin.

(1) *Isolierung*: — Die Pulver von getrockneten, japanischen Tabakblättern werden mit Calciumcarbonat gemischt und mit 95 proc. Alkohol warm extrahiert. Der alkoholische Auszug, nach Entfernung des Wachses, welches beim Erkalten sich abscheidet, wird in Vakuum eingedampft, der Rückstand mit Wasser behandelt und filtriert. Die wässrige Lösung wird mit neutralem Bleiazetat gefällt, der Niederschlag mit verdünnter Schwefelsäure zersetzt und stark eingeeengt. Die Lösung, nach wiederholter Extraktion mittels Aether, wird erschöpfend mit Essigäther heiss extrahiert und der nach Eindampfen des Essigäthers gewonnene Sirup in einer kleinen Menge Wasser gelöst. Beim längere Stehenlassen scheiden sich hellgelbe, körnige Aggregate ab, die durch Umkrystallization aus verdünntem Alkohol lange, blassgelbe, glänzende Nadeln liefern, welche ich Tabacinin genannt habe. Ausbeute, 0.01~0.03%.

(2) *Eigenschaften*: — In kaltem Wasser oder Alkohol ist Tabacinin schwer, in heissem Alkohol leicht, aber Äther unlöslich. Ferrichlorid gibt grüne Färbung; Bleiazetat verursacht einen gelben Niederschlag. Mit gelber Farbe wird Tabacinin von konzentrierter Schwefelsäure gelöst. Tabacinin beginnt beim Erhitzen auf 175°C sich bräunen und zersetzt sich bei 227°C unter Gasentwicklung.

(3) *Hydrolyse*: — 2 g. Glykosid werden in 200 c.c. 5 % schwefelsäurehaltigem, verdünntem Alkohol suspendiert, 5 Stunden gekocht, dann der Alkohol abdestilliert und Wasser zugefügt. Die ausfallenden, gelben, nadelförmigen Krystalle werden abfiltriert und aus 50 proc. Alkohol, umkrystallisiert.

(4) *Glucuronsäure*: — Die Hydrolyseslösung, nach Entfernung des Aglycon, wird mit Bariumcarbonat neutralisiert, das Filtrat eingedampft und der weisse, amorphe Rückstand in einer kleinen Menge Wasser aufgenommen. Die wässrige Lösung ist Orzin-salzsäure- und Phlorogluzinsalzsäure-reaktion positiv; reduziert Fehlingsche Lösung; durch Schwefelsäure fällt Bariumsulfat aus.

Glucuronsäurephenylosazon: Smp. 198°C, 15.03% N (Ber. 15.05% N)

Leicht löslich in Aceton, beim Verbrennen bleibt keine Asche zurück.

p-Brom-phenylosazonglucuronsaures Barium: Smp. 215°C. Beim Glühen bleibt sich weisse Asche zurück.

### (B) Tabacilin.

(1) *Isolierung*: — Das Filtrat vom Bleiniederschlag, der Tabacinin enthält, wird nach Entbleien mit verd. Schwefelsäure, unter vermindertem Druck zu dickem Sirup eingedampft der Rückstand mit Chloroform extrahiert, der durch Abdestillation des Chloroforms erhaltene Sirup wird wiederholt mit grösseren Mengen Petroleumäther behandelt und so die darin lösbaren Substanzen vollständig beseitigt werden. Wenn dieser Sirup in Alkohol gelöst und wieder mit Petroleumäther gefällt wird, so erhält man einen hellbraunen, wachsartigen Glykosid, der von Verfasser als \**Tabacilin* bezeichnet werden ist. Ausbeute, 0.4~0.5%.

(2) *Eigenschaften*: — Tabacilin ist sauer; löst sich leicht in Alkohol und Chloroform; schwerer in Wasser, Äther, Essigäther und Benzen. Durch 2 proc. kaltes Kaliumhydroxyd gibt Tabacilin keine Zucker, aber kochende verdünnter Mineralsäure spaltet es in Glykose, Nicotin und andere Bestandteile.

(3) *Hydrolyse*: — 30 g. Tabacilin wird in 200 c.c. Alkohol gelöst, 200 c.c. 6 proc. Schwefelsäure zugefügt und 10 Stunden zum Sieden erhitzt. Die Flüssigkeit wird eingedampft, mit Wasser verdünnt und mit Äther ausgeschüttelt. Wird aus den ätherischen Extrakten der Äther abdestilliert, so erhält man einen sauerreagierenden Rückstand.

(4) *Nicotin*: — Eine Portion der Hydrolyseslösung wird mit Sodalösung schwach alkalisch gemacht und mit Petroleumäther ausgezogen. Die nach Verdampfen der Petroleumätherlösung zurückbleibende Flüssigkeit hat Nicotingeruch; reagiert alkalisch und färbt sich durch p-Dimethylaminobenzaldehyd und Salzsäure rot.

Nicotindipikrat: — Smp. 219°C, 18.23% N (Ber. 18.05% N).

Nicotinchlorplatinat: — Smp. 34.20% Pt (Ber. 34.09% Pt).

Nicotinchloraurat: — 46.64% Au (Ber. 46.79% Au).

Um weiter festzustellen dass, Nicotin keine Verunreinigung, sondern ein Bestandteil des Glykosids ist, wird Tabacilin in verdünnter Schwefelsäure gelöst und wieder mit Chloroform extrahiert. Das aus der Chloroformlösung, wie oben erwähnt, dargestellte Glykosid gibt Nicotin bei Hydrolyse.

(5) *Glykose*: — Der grössere Teil der Hydrolyseslösung, nach Extraktion mittels Äther, wird mit Bariumcarbonat neutralisiert. Das Filtrat wird eingedampft und der hinterbleibende Sirup, nach Entfernung des Nicotins durch Ausschütteln mittels Petroleumäther, mit warmen Alkohol behandelt. Die alkoholische Lösung wird abgedampft, der Rückstand in Wasser aufgeno-



mmen neutralisiert mit Barytwasser und dann Bleiazetat und Bleiessig gefällt. Das Filtrat vom Bleiniederschlag wird mit verdünnter Schwefelsäure zersetzt. Diese wässrige Lösung reagiert auf die Seliwanoffsche Reaktion negativ; reduziert Fehlingsche Lösung; gibt kein schwerlösliches Phenylhydrazon.

Glykosephenylosazon: — Smp. 207°C, 15.57% N (Ber. 15.64% N).

Glykose-p-bromphenylosazon: — Smp. 212°C, Diese Osazone, bei der Mischprobe, zeigen keine Schmelzpunktsdepression.

### Zusammenfassung.

(1) Aus den Tabakblättern wurden zwei neue Glykoside isoliert, die Tabacinin und Tabacilin genannt worden sind.

(2) Bei Hydrolyse, liefert Tabacinin Glucuronsäure und ein gelbes Kristall.

(3) Unter den Hydrolysesprodukten des Tabacilins wurden Nicotin und Glykose nachgewiesen.

## On the Mineral Constituents of American "Loess", a Soil of Aeolian Deposit.

By

SOJIRO KAWASE and YOSHIJIRO KIHARA.

(Received December 26, 1931).

There are three regions known in the world, where aeolian soils, known as "Loess", are widely distributed, i.e. Central China, Central Europe and the northern part of the U. S. A. The mineral composition of "Loess" of China and of Europe is already well investigated, but that of the U. S. A. is not yet described in any literature.

On his tour through America in 1923, Kawase, one of the authors, visited the Soil Bureau in the U. S. Department of Agriculture at Washington, and inquired about the composition of American Loess, but he could get no information concerning this question. So he went to Kansas City where the thick deposit of this aeolian soil is abundantly found and he took a few samples of the soil from a railway-cut in the neighbourhood of the city. Those samples were brought to Japan and afterwards studied.

The air-dried Loess is pale yellowish brown in color and contains some "Loesskinder".

Powdered between fingers, the particles of the soil were found to be less

than 2 m.m. in diameter. It can be divided into the following two parts by Sikorski's method :

Sandy part (2~0.05 m.m.) 27.81%, Clayey part (< 0.05 m.m.) 72.19%.

Of the sandy part, the greater portion, about 98% of it, consists of particles less than 0.5 m.m. in diameter.

Then we investigated the mineral constituents of the air-dried sample by means of a polarization-microscope, under the kind guidance of Dr. T. Wakimizu, formerly Professor of Geology in the Tokyo Imperial University.

The result is as follows: This soil consists of quartz, feldspar, hornblende, zirkon and magnetite. (Fig. I and II).

In the Figure I, H denotes hornblende; Z, zirkon, and M, magnetite respectively, and the non-colored mineral constituents, such as quartz and feldspar are not denoted any way. Among these constituents, mentioned above, quartz occupies the greatest portion and magnetite and zirkon the smallest. Mica and augite could not be found in the sample.

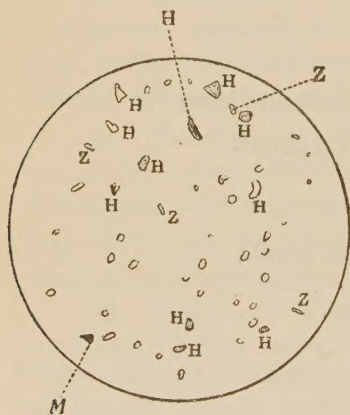


Fig. I

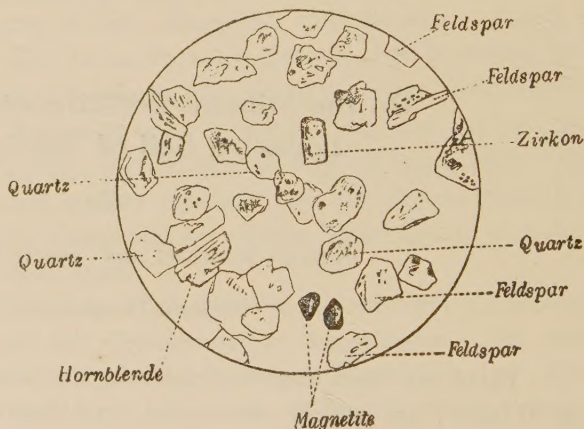


Fig. II

## Iodine Contents of Important Agricultural Product in Japan.

By

K. SHIRAHAMA and G. SHIMIZU.

(Received November 26, 1931).

Iodine contents were determined by the method of Leitch and Henderson



somewhat modified by the author. Results are as follows :—

Sample	Moisture percent	Iodine ( $\gamma$ ) per 100 g. of fresh sample.	Iodine ( $\gamma$ ) per 100 g. dry matter
Rice	15.40	7.8	9.2
Spring wheat	13.63	22.3	25.7
Winter wheat	14.01	24.5	28.5
Winter rye	13.50	15.0	17.4
Barley (Chevalier)	13.44	19.6	22.6
Barley (Imperial)	12.28	29.3	33.7
Naked barley	12.88	17.0	19.6
Corn	13.92	66.4	77.2
Italian millet	12.95	4.8	5.5
Barnyard millet	11.38	6.9	7.7
Sorghum	14.15	17.5	20.4
Common millet	12.39	14.9	17.0
Soy bean	12.37	47.0	53.6
Azuki bean	14.18	18.6	21.7
Green pea	14.32	64.9	75.7
Cherry	77.86	5.6	25.2
Tomato	93.80	5.1	31.5
Apple (Hōgyōku)	88.02	9.5	86.2
Apple (No. 9)	84.50	3.9	25.3
Spinat	88.67	12.1	106.4
Caboge	91.22	13.5	154.0
Potato	81.04	16.9	89.2
Carrot	83.98	9.8	61.1
Turnip	91.01	6.9	76.2
Onion	91.95	6.5	81.2
Lily	69.46	3.7	12.5
Cucumber	96.35	3.6	99.4
Egg-plant	92.94	5.6	79.0

Our result show that the products in Japan contain 20~80 times as much as European products which were reported by Mayrhofer and Waisitzky, Bleyer, Fellenberg, and McClendon and Remington.

## The Protein Requirement of Growing Chicks. (Part I.)

By

KOZO SUZUKI and TADASHI HATANO.

(Received November 26, 1931).

In order to find the relative effects of variations in the levels of protein

in the rations upon the growth rate of chicks, upon the age of laying the first egg, upon the body weight at maturity of the female and upon the development of several organs, 180 Single Comb White Leghon day-old chicks, which were hatched in the same incubator, were divided into 3 equal lots, designated as Lot A, B and C, each consisting of 60 chicks.

The following 3 kind of rations were given to each lot separately through all growing period of chicks from day after hatching to 25 weeks of age.

	Lot A	Lot B	Lot C
Rice refuse	10%	10%	10%
Yellow corn	30	30	30
White corn	25	30	35
Rice bran	15	15	15
Fish meal	15	10	5
Salt mixture	5	5	5

Salt mixture consists of 3 parts of bone-meal, 1 part of ground oyster-shell and 1 part of common salt by weight.

The compositions of these rations were as follows :—

	Moisture	Crude protein	Crude fat	Crude fiber	N-free extract	Crude ash
Ration of Lot A	11.62%	18.27%	6.93%	2.39%	51.63%	9.16%
" " B	11.39	15.58	6.61	2.54	55.28	8.60
" " C	11.51	12.85	6.29	2.64	58.62	8.04

The result of all trials in this experiment may be summarized as follows :

All the chicks of two lots, Lot B and C, which received the rations of low protein level, 15.58 and 12.85 percent protein, suffered from heavy diarrhoea and many of them died during the first few weeks of the growing period.

It is necessary to feed a ration relatively high in protein during the first five weeks. A lower protein level than 15.85 percent in the ration is not sufficient for normal growth during the first 10 or 12 weeks of age. It seems that 13 percent protein in the ration may be sufficient for the growing chicks after the first 10 or 12 weeks and until maturity.

There is no effect of the different levels of protein in the rations which were given during the whole growing period of chicks upon the age of laying the first egg.

There is no influence of the different levels of protein in the rations during the growing period upon the development of several organs (liver, kidney, spleen, pancreas, adrenal body, pituitary body, thymus gland and testicle) except for the thyroid gland.



## Investigation on Cellulose Decomposition in Soils. (*Continued*)

### II. Detection and Estimation of Number of Principal, Aerobic Cellulose Decomposing Bacteria.

By

ARAO ITANO and SATIYO ARAKAWA.

(Received December 2, 1931).

Twenty two samples of air-dried soils, twelve fresh soils and three composts were investigated as to the detection and estimation of number of principal, aerobic cellulose decomposing bacteria by using synthetic cellulose liquid medium after Dubos.

*Cytophaga hutchinsoni* was found in about 86% soils examined, and in case of the fresh soil, 1,000~100,000 of them were present in 1 g. sample.

In 1 g. of fresh soil of which the reaction was pH (7.0~8.0) 1,000~10,000 of *Cellvibrio* type bacteria were found but their distribution is limited since they are influenced by the reaction of soil to a great extent.

Although 1,000~110,000 of aerobic cellulose decomposing bacteria were present in 1 g. of fresh soil, they decrease in number to one to ten thousandth when the soil is air-dried. However qualitatively no change was observed.

It was not possible to obtain any information on the anaerobic cellulose decomposing bacteria by the method employed in this investigation.

Further investigation of the bacteria which were isolated is in progress.

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## On The Natural Pigments of Raw Silk Fibre of the Domestic Cocoon. (Part III).

Melting Point of Cocoon Xanthophyll.

By

MASAMI OKU.

(From the Chemical Laboratory of Gunze Raw Silk Mfg. Co., Ltd.,  
Ayabe-machi, Kyôto-hu, Japan.).

(Received December 25, 1931).

In the previous papers I have reported that the main natural yellow pigment of domestic yellow cocoons was xanthophyll, derived biologically from

mulberry leaves upon which silk worms feed and determined its melting point as 175°C (uncorr.).

Recently, xanthophyll was defined by R. Kuhn as the name given to carotinoid group which contains hydroxyl groups and 40 atoms of carbon and he reported that the true melting point of xanthophyll as a chemical entity higher than hitherto usually taken into account.

I have prepared xanthophyll of yellow cocoons and mulberry leaves by the processes given in previous papers once again and purified in according to R. Kuhn by distributing between petroleum ether and 85% methanol, then recrystallizing from mixed solution of methanol and ethyl ether and determined its melting point which showed 193°C (corr.).

As this melting point coincides exactly with that of lutein (leaf xanthophyll), the xanthophyll of yellow cocoons must be lutein and the melting point of xanthophyll formerly reported as 175°C (uncorr.) should be corrected as 193°C (corr.)

## Studies on Agar-agar. II.

On the Two Kinds of Hydrato obtained from Hot-water-hydrolysis.

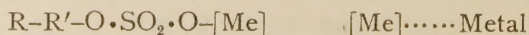
By

E. TAKAHASHI and K. SHIRAHAMA.

(Received December 26, 1931).

Two kinds of hydrato were obtained by hot-water-hydrolysis, the one being insoluble in cold water (Hydrato-Kanten- $\delta$ ) and the other soluble in cold water and precipitated by alcohol from the filtrate of the above (Hydrato-Kanten- $\lambda$ ).

$\delta$  seems to be a simple polysaccharide containing almost no ash and is very easily decomposed by dilute mineral acids, however,  $\lambda$  contains much ash and not easily decomposed. Ash composition of  $\lambda$  consisted almost of  $\text{SO}_4$  and Ca, and a small amount of Mg, Na and K etc. Through electro-dialysis  $\text{SO}_4^{--}$  appears slightly in the anode while  $\text{Ca}^{++}$  removed to the cathode very conspicuously and after the electro-dialysis the  $\lambda$  solution becomes distinctly acidic. From the above points, the chemical structure of agar is considered as follows:—



In the above formula, the linkage between R and R' is considered to be rather related to jelly formation, though Samec and Isajevič explained that the jelly formation of agar is due to the linkage between R' and  $\text{SO}_4$ .



### On the Gluconic Acid Fermentation. (Part III.)

On *Bacterium Hoshigaki* var *glucuronicum* I. nov. spec.  
(*Bacterium industrium* var *Hoshigaki* nov. spec.).

By

TEIZO TAKAHASHI and TOSHINOBU ASAI.

(Received January 12, 1932).

*Bacterium Hoshigaki* var *glucuronicum* I. nov. spec. This bacterium was once described under the name of *Bact. industrium* var *Hoshigaki*\*. It is shot rod with round end and generally occurs in single but seldom in pairs or often in chains, 1.5~8.0  $\mu$  long, 0.5~0.8  $\mu$  thick. It is motile and has vigorous tendency to form involution form at room temperature. The optimum temperature for the growth was 25~26°C and the same for the acid production was 24~25°C. The death of this organism was perceived in 5 minutes at 50°C. It forms acid from arabinose, glucose, fructose, galactose, sucrose, maltose, lactose, raffinose, dextrine, starch, inulin, glycol, ethyl alcohol, propyl alcohol, glycerine, mannite, sorbite but not from methyl alcohol. It forms rosy red coloured growth in the medium containing such poly-alcohols as glycol, glycerine, mannite and sorbite.

It makes growth in the solution containing 50% of glucose but not in solution of 55% of the sugar. It could not grow in solution containig 10% of ethyl alcohol. It does not oxidize acetic acid as experienced by *Bact. Hoshigaki* var *rosea*. It makes no growth in Beijerinck's solution but inverts cane sugar, so that it could not belong to *Bact. aceti* Pasteur or *Bact. rancens* group after Hoyer's system. From Hermann's coccus viz; *Bact. gluconicum*, this one differs by the property of giving film in fluid media, forming involution forms and acidifying maltose, lactose, starch and inulin. It produces 10.38% of gluconic acid in yeast water containing 10% glucose. This organism produces oxygluconic- and glucuronic acid in presence of Ca-carbonate in the nutrient solution.

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\* Nippon Nōgei kwagaku Kwaishi, Vol. 6 No. 6, (1930).

### On Gluconic Acid Fermentation. (Part IV.)

On Bact. Hoshigaki var glucuronicum II and III nov. spec.

By

TEIZO TAKAHASHI and TOSHINOBU ASAI.

(Received January 12, 1932).

In the first communication (Nippon Nōgei Kwagaku Kwaishi Vol. 6. No. 3. March. 1930) on gluconic acid fermentation by authors there was mentioned two acetic bacilli named provisionally Bact. B and C. They were new species in every point of view and their characters are described below.

*Bacterium Hoshigaki var glucuronicum II. nov. spec. (Bact. C.)*

It is short rod,  $0.8\sim3.6\ \mu$  long and  $0.5\sim1.5\ \mu$  thick.

Single or in pairs and motile. It forms viscous film in fluid media, which alters to turbid. The occurrence of involution form is rare. The optimum temperature for the growth is  $30\sim31^{\circ}\text{C}$ , but the same for the acid formation is  $26\sim28^{\circ}\text{C}$ .

The death comes at  $55^{\circ}\text{C}$  in 5 minutes. It forms acid from arabinose, glucose, fructose, galactose, sucrose, raffinose, glycerine, mannite, sorbite, ethyl alcohol, propyl alcohol, but not from maltose, lactose, dextrine, starch, inulin, methyl alcohol.

In nutrient solution containing 55~60% of glucose it grows well, the property differing from Bact. Hoshigaki var rosea or Bact. industrium var Hoshigaki (Bact. Hoshigaki var glucuronicum I). It makes growth in solution containing even 12% of ethyl alcohol. It oxidizes acetic acid against to Bact. Hoshigaki var glucuronicum III. It makes no growth in Beijerinck's or Pasteur's solution but could assimilate inorganic nitrogen (ammonium-salt) in Fuhrmann's or Henneberg's second solution. By this property this one differs from Bact. Hoshigaki var rosea and Bact. Hoshigaki var glucuronicum I. But it could not assimilate nitrogen of urea in Henneberg's solution.

It inverts sucrose as Bact. aceti Pasteur group or Bact. xylinum Brown group do. It produces 8.9% of gluconic acid in 10% solution of glucose, but in the solution containing peptone (0.1%) or ammonium phosphate (0.3%) and 10% of glucose the production of gluconic acid increases up to 10%.

*Bacterium Hoshigaki var glucuronicum III. nov. spec. (Bact. B)*

It is short rod of the size of  $0.8\sim2.0\ \mu \times 1.2\sim4.0\ \mu$ , but generally  $1.0\sim1.5\ \mu \times 1.5\sim3.0\ \mu$ . Single, or pairs and motile. It forms viscous film and bacteria ring in fluid media. Involution forms occur rarely, but in glucose yeast



water or Henneberg's solution occur in abundance and even in solid media they occur in seldom.

The optimum temperature for the growth and acid formation is quite same as *Bact. Hoshigaki* var *glucuronicum* II. This organism inverts sucrose as *Bact. aceti* Pasteur group or *Bact. xylinum* Brown group do. It forms acid from arabinose, glucose, fructose, galactose, sucrose, ethyl alcohol, propyl alcohol, but not from maltose, lactose, raffinose, glycerine, mannite, sorbite, dextrine, starch, inulin and methyl alcohol.

It could not grow in solution containing 50% of glucose, or 5% ethyl alcohol. It could not oxidize acetic acid. It could assimilate inorganic nitrogen (ammonium nitrogen) in Fuhrmann's or Henneberg's IIInd solution, although it could not grow in Beijerinck's or Pasteur's solution. By this property this one differs from *Bact. Hoshigaki* var *rosea* and *Bact. Hoshigaki* var *glucuronicum* I. It could assimilate nitrogen of urea against to *Bact. Hoshigaki* var *glucuronicum* II or *Bacterium Hoshigaki* var *rosea*. It forms 7.65% gluconic acid in the solution containing 10% of glucose.

*Bact. Hoshigaki* var *glucuronicum* II and III also produce oxygluconic- and glucuronic acid in presence of calcium carbonate in the nutrient solution.

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### Isolation of "Oryzanin" (Antineuritic Vitamin) from Rice-polishings. (First Report.).

By

SATOR OHDAKE.

(*Agricultural Chemical Laboratory, Tokyo Imperial University,  
Faculty of Agriculture, Komaba, Tokyo.*)

(Received January 18, 1932).

In 1911, Dr. U. Suzuki<sup>(1)</sup> isolated an active substance which cures pigeons from polyneuritis, from the alcoholic extract of rice polishings. He proposed the name "Oryzanin" to it and concluded with the view that Oryzanin is a new and hitherto overlooked food factor, essential for animal nutrition beside

- 
- (1) U. Suzuki: On an antineuritic substance of rice-bran 1st Rep. J. Chem. Soc. Tokyo. Vol. 32, 1 (1911), 4-17; II. rep. Ibid. Vol. 32, 2 (1911); III. rep. Ibid. Vol. 32, 13 (Apr. 1911); IV. rep. Ibid. Vol. 32, 9 (1911), 874-899; V. rep. Ibid. Vol. 33, 2 (1912), 113-130; VI. rep. Ibid. Vol. 34, 9 (1913), 1123-1125.  
U. Suzuki, T. Shimamura and S. Ohdake: Über Oryzanin, ein Wirkamer Bestandteil der Reiskleie und seine physiologische Bedeutung. Bioch. Z. 43 1-2, (1912), 89-153; J. Coll. Agr. Tokyo. Vol. 4, (1913).

proteins, fats, carbohydrates and mineral matters. A numbers of valuable studies<sup>(2)</sup> on vitamin were reported thereafter.

Recently, Jansen and Donath<sup>(3)</sup> reported that they succeeded in isolating the antineuritic substance in crystalline state from rice polishings. The process used by them is shown in the following schematic table I.

They stated the active substance to be designated by the formula  $C_6H_{10}N_2O$  and its hydrochloride  $C_6H_{10}N_2O-HCl$  forms needle crystals, melting at  $250^\circ C$ , soluble in water and methyl alcohol but insoluble in cold absolute alcohol and acetone. It gives a preceipitate by mercuric chloride, mercuric sulphate and Dragendorf's reagent and also gives intensive Pauly's diazo-reaction. They claimed the protective dose of the hydrochloride to be 0.002 mg. daily for a bondol and 0.03~0.06 mg. for a pigeon.

The present author worked previously on the chemical constituents of yeast extract<sup>(4)</sup> since 1924 and isolated adenin, cholin, hypoxanthin, leucin, nicotinic acid, thymine, thyrosin, xanthin, adenylythiomethylpentose, methionin, two unknown bases  $C_6H_8N_2O$  &  $(C_3H_6N_2)_n$  etc. but failed to isolate the active substance in pure state. Furthermore the scanty supply of fresh brewers yeast from the brewery forced the author to apply rice-polishings as the material and the antineuritic preparation "Active Oryzanin" was prepared which cures pigeons from polyneuritis in the dose of 3~4 mg. daily.

Soon after the appearance of Jansen and Donath's publication, the present author tried the same experiment starting from "Active Oryzanin" following the dutch worker's process with minor modifications, and the hydrochloride of antineuritic substance was isolated in crystalline state beside adenin, hypoxanthin, nicotinic acid, cholin, two unknown bases  $(C_3H_6N_2)_n$  &  $C_8H_{10}NO_2$  etc. The antineuritic substance isolated by the author is the hydrochloride of a new

- (2) C. f. Literatures on vitamin: H. C. Sherman & S. L. Smith: The Vitamins; R. Berg: Die Vitamin; C. Funk: The vitamines; W. H. Eddy: The vitamine manual; E. V. McCollum: The newer knowlege of nutrition; U. Suzuki: Chemical stud es of vitamin B in Japan, Scientific papers of Institute of physical and chemical research, Vol. 4, 295~302, (1926).
- (3) B. C. P. Jansen and W. F. Donath: Isolation of antiberiberi-vitamin; Mededelingen van den Dinst der Volksgezondheid in Ned-indie, Anno, (1927), Part 1 (1927).
- (4) S. Ohdake: On the Chemical constituents of yeast-extract, J. Agr. Chem. Soc. Japan, Vol. 3 7~8 (1927) 98~121; II rep. Ibid. Vol. 7 10 (1931), 859~878.  
U. Suzuki, S. Ohdake and T. Mori: On the occurrence of a new sulphur compound in yeast, J. Agr. Chem. Soc. Japan, Vol. 1 2 (1924), 127~136; Bioch. Zeit. B 151 II 3~6 (1924), 278~289.  
S. Ohdake: On the occurrence of a sulphur containing aminoacid in yeast, J. Agr. Chem. Soc. Japan, Vol. 1 8 (1925), 601~609; Bioch. Zeit. B. 161 II 4~6 (1925), 446~455.  
S. Ohdake: On the distribution of a new thioamino acid, Jour. Agr. Chem. Soc. Japan, Vol. 2 10 (1926).



sulphur compound having the formula  $C_{12}H_{16}N_4SO_2$ . It crystallised in long monoclinic plates melting at  $250^\circ\text{C}$  (uncorr.) and its curative dose for a pigeon and a white rat was 0.01–0.02 mg. daily.

Table I. Isolation of Antiberiberi-Vitamin.

(by Dr. B. C. P. Jansen & W. F. Donath, Medical Laboratory, Weltevreden, Java, Dutch-east-Indies, (1927).

Rice—Polishings, 100 kg. (Portective dose for Bondol 0.1—0.14 g.)	
+ 300 L $H_2O + H_2SO_4 + \text{Alcohol}$	
Water-Extract,	
Acid-clay..... 3 kg.	
+ $Ba(OH)_2$ Aq. sol. (PH 12—13)	
+ $H_2SO_4$	
Vitamin-Solution, 4 L. (Solid, 100 g.) PH. 2—3.	
↑ $AgNO_3$ .....50 g. PH 2—3	
—ppt. (I)	
$Ba(OH)_2$ PH 4.5	
—ppt. (II)	
$Ba(OH)_2$ PH 6.5	
—ppt (III) —HCl	
$Ba(OH)_2$ PH 8.0	
—Filt.	
ppt. (IV)	
	Solid.....20 g. Activity.....0.08 mg. for Bondol
	Phosphotungstic acid Solid.....5 g. Activity.....0.03 mg. for Bondol.
	Alcohol absolute —Sol— $PtCl_4$ ppt— $H_2S$ —Alcohol absolute
	Acetone
	Crystals
	Vitaminhydrochloride $C_6H_{10}N_2O-HCl$ Cryst.....needle M. P. t. .... $250^\circ\text{C}$ . React.....Diazo-reaction, Activity...0.002 mg. for Bondol, 0.03—0.06 mg. for Pigeon.
	Gold double Salt :— Cryst.....needle $C_6H_{10}ON_2HCl \text{ AuCl}_3$
	Picolonate :— Cryst.....needle M. P. t. .... $165^\circ\text{C}$ .

## Experimentals.

*Preparation of "Active Oryzanin":*— Each 100 kg. of fresh rice-polishings were macerated with 800 litres of 0.3% sulphuric acid. After continual stirring for about 3 hours, the mixture was centrifuged and filtered. To the filtrate 5~7 kg. of finely powdered acid-clay was added and stirred for a few hour. The acid-clay adsorbes the greater part of the active substance and subsides

to the bottom, the supernatant fluid was syphoned off. After centrifuging, the sediment was collected and washed with water acidurated by sulphuric acid. To liberate the active substance again, the activated clay was then extracted with 150 litres of 0.5% baryta water and filtered. The second extraction was repeated with 100 litres in the same manner. The united filtrate, freed from an excess of baryta by sulphuric acid, was concentrated in vacuum to a small volume and the requisite quantity of strong alcohol was added to make the alcoholic content of the solution 80% by volume. A voluminous precipitate which consists of proteins and other impurities, was settled and filtered off. When the alcoholic filtrate concentrated to a syrupy consistence containing about 30% of water, the antineuritic concentrate so-called "Oryzanin-extract" was obtained. The yield was about 250 g. and its antineuritic activity was in daily doses of 12 mg. for pigeons (Chart. 1), therefore about 83% of the active

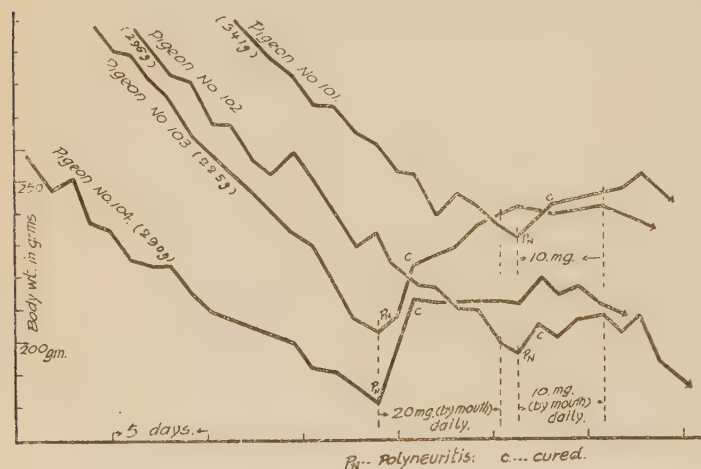


Chart. 1—Pigeons on polished rice, & Oryzanin-Extract.

to make 5% to the solution, then a 50% aqueous solution of phosphotungstic acid was added. The precipitate which was collected by suction after standing 24 hours, decomposed with baryta in the usual manner. The filtrate freed from an excess of baryta by sulphuric acid, evaporated in vacuum to about 250 g. and 2 litres of 95% alcohol were added to make the alcoholic content of the solution 85% by volume, whereby a voluminous precipitate separated out which were filtered off. The alcoholic filtrate was evaporated in vacuum to dryness. In this way, a light brown mass so-called "Active Oryzanin" was obtained. The yield was about 33 g. i.e. adequate to 0.03~0.04% of the original rice polishings.

*Biological experiment with this substance resulted as follow:—*

- (1) When pigeons suffering from polyneuritis by exclusive feeding on

substance contained previously in the original rice-polishings was carried to this concentrate.

For further concentration 250 g. of "Oryzanin extract" was now dissolved in 2 litres of water and added with the requisite quantity of sulphuric acid



polished rice, were supplemented daily with 4 mg. of the preparation, they were cured perfectly in a day, improving the symptoms in 4~5 hours. (Chart. 2).

(2) Pigeons, when fed on the synthetical diet consisting of 71% purified starch<sup>(5)</sup>, 15% extracted meat (so-called meat-protein)<sup>(6)</sup>, 10% butter<sup>(7)</sup>, 4% McCollum salts-mixture<sup>(8)</sup> and administered with 4 mg. of active oryzanin daily, re-

mained entirely health for 50 days against polyneuritis, while symptoms developed after a week by the removal of the supplement. (Chart. 3).

(3) Young rats, about 45 g. in weight when developed the symptoms of neuritis by feeding on the synthetical diet, consisting of 70% purified starch, 15% meat-protein, 10% butter, 5% McCollum's salts mixture and with the



Chart. 2—Pigeons on polished rice & Active-Oryzanin.

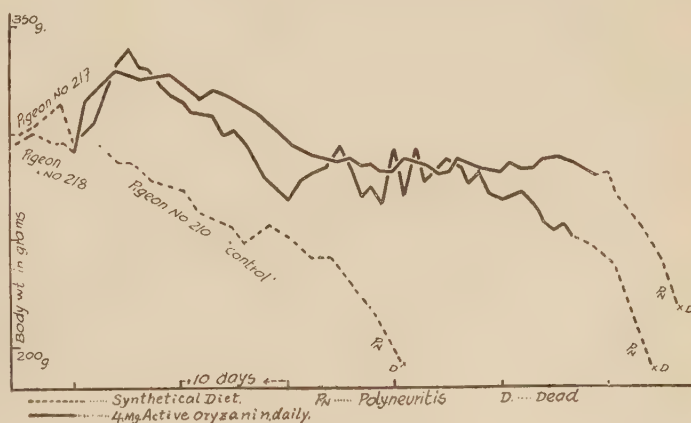


Chart. 3—Pigeons on synthetical diet, & Active Oryzanin.

- (5) Purified starch is prepared by extracting "Starch J. P. C." with 80~90% alcohol repeatedly to make free from vitamins and then with ether.
- (6) Milled fresh meat is extracted by boiling with enough quantity of water repeatedly and with 80~90% alcohol, then with ether. Extracted meat, prepared by this way, is free from B<sub>1</sub>, but still contains the substance which promotes rats-growth when supplemented by antineuritic vitamin.
- (7) Butter is purified to make free from B<sub>1</sub>. by shaking with warm-water repeatedly and oily layer is collected after centrifuging.
- (8) [McCollum's salts mixture, No.185], E. V. McCollum, N. Simonds & W. Pitg: Journ. Biol. Chem. **29** (1917), 521. (Comp. NaCl 0.173...MgSO<sub>4</sub> 0.266...NaI<sub>2</sub>PO<sub>4</sub>+H<sub>2</sub>O 0.347...K<sub>2</sub>HPO<sub>4</sub> 0.954...CaH<sub>4</sub>(PO<sub>4</sub>)<sub>2</sub>+H<sub>2</sub>O 0.540...Fe-Citrate 0.118...Ca-I lactate 1.300.)

addition of 3 drops of Codliver oil daily, were cured quickly and grew normally by the supplement of 4 mg. daily. (Chart 4.)

(4) Young rats, fed on the same diet as above, supplemented with 4 mg. of active oryzanin daily from the begining of the test, were protected from neuritis and grew perfect healthy. (Chart. 5).

(5) Its pigeon-curative day-dose<sup>(9)</sup> resulted to be of the order of 0.75 mg ;

Pigeon No.	Dose, injected mg.	Days, protected	Day-dose, average mg.
302	5.0	6	} 0.75
303	5.0	8	
304	5.0	7	
304	5.0	6	

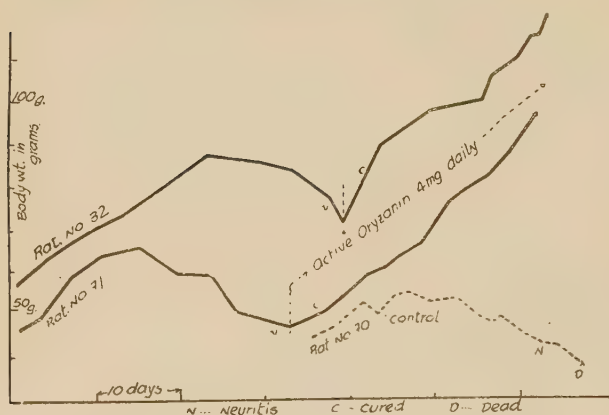


Chart. 4—Rats on synthetical diet, & Active Oryzanin.

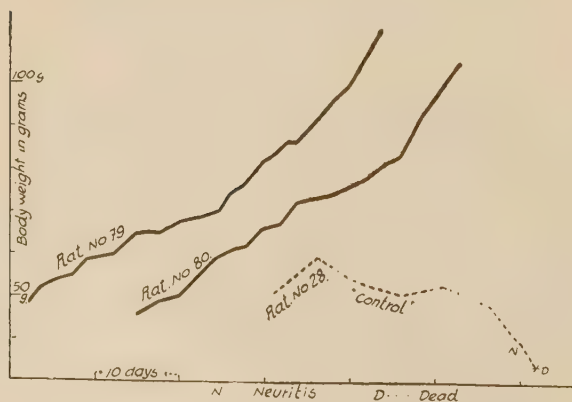


Chart. 5—Rats on synthetical diet, & Active Oryzanin.

From above results, it is ascertained, its anti-neuritic activity to be in daily doses of 4 mg. for pigeons as well as for white rats and its pigeon curative day-dose to be of the order of 0.75 mg.

(6) White rats, fed on the synthetical diet<sup>(10)</sup> which consisted of 60% purified starch, 20% purified casein, 15% peanuts oil (in place of cottou seed oil), 5% McCollum salts mixture and with the addition of 3 drops of cod-liver oil daily, exhibited the symptoms of neuritis and died after 4 weeks decreasing body weight. (Chart. 6~A).

Rats, fed on the same diet supplemented with 4 mg. of active oryzanin daily, was protected from neuritis but the

(9) Kinnorsly and Pelters: Biochem. J., **19** (1925), 820.

(10) Chick and Roscoe: Bioch. J., **22** (1928), 790.

symptoms of pellagra appeared after 5 weeks without promoting growth. (Chart. 6~B).

(7) White rats fed on the same diet as above, supplemented with 0.4 g. of autoclaved yeast daily, exhibited the symptoms of neuritis after 4 weeks, but rats grew normally against neuritis and pellagra when supplemented with both 4 mg. of active oryzanin and 0.4g autoclaved yeast simultaneously. (Chart. 7).

These results show clearly the lack of pellagra protective and growth promoting factors in active oryzanin.

For the isolation

of antineuritic substance and the allied compounds, active oryzanin was fractionated into five fractions by silver nitrate and baryta. The following account represents a standardised treatment of a batch or 100 g. of "active oryzanin" and five batches were brought together for a lot.

*Fractionation by silver nitrate and baryta*:— 100 g. of "active oryzanin" were dissolved in 4 litres of water and added with about 15 g. of sulphuric acid to bring the  $P_H$  to 2.5~2.6. A small quantity of resinous substance, thereby separated out, was filtered off. The clear filtrate, brought into a hard-glass beacker of about 10 litres contents, was stirred continuously by means of electric driving and then added with 200 c.c. of 50% aqueous solution of silver nitrate that showed a slight excess. The precipitate by silver nitrate, allowed to settle for 2~3 hours, filtered by suction and collected on a porous-plate after washing with about 300 c.c. of water. About 25 g. of a dark colored precipitate were obtained which showed to be inactive.....This is called "Fraction I".

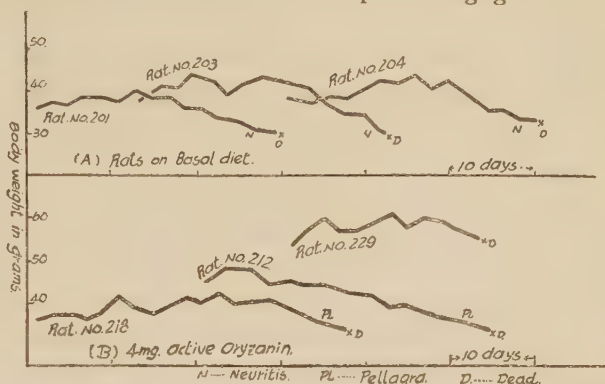


Chart. 6—Rats on synthetic diet, & Active Oryzanin.

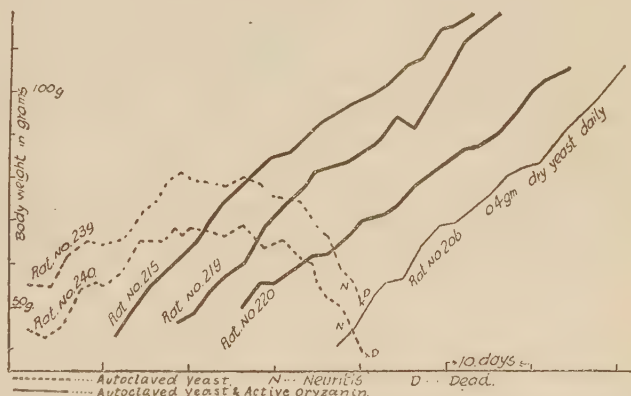


Chart. 7—Rats on synthetic diet, & autoclaved yeast.



The filtrate from the "Fraction I" was then treated gradually by constant stirring with the requisite quantity of 4% baryta water to bring the  $P_H$  to 4.5 and allowed to settle for 2~3 hours. The precipitate which contained chiefly nicotinic acid, was filtered by suction. About 30 g. of a dark gray precipitate were obtained....."Fraction II".

The filtrate from the "Fraction II" contains the active substance, and its  $P_H$  was brought to 6.8 by further addition of requisite quantity of baryta water with constant stirring. The dark brown precipitate which carried greater parts of the active substance, was filtered by suction after standing over night. The yield was about 50 g. in average....."Fraction III".

More baryta water was then added to the filtrate from the "Fraction III" in the same manner as to the previous fraction to bring the  $P_H$  to 9.0, and the precipitate formed thus, was filtered after settling down for a few hours. About 50 g. of a dark brown precipitate were obtained which contained still a small quantity of active substance....."Fraction IV".

The filtrate was then treated with hydrochloric acid and sulphuric acid to remove an excess of silver and baryta. The acidity of the solution, having been measured by titration, was brought 5% of sulphuric acid by adding a requisite quantity of sulphuric acid to the filtrate, then a 50% aqueous solution of phosphotungstic acid was added. After standing for about 24 hours, the precipitate was filtered by suction and washed with 5% sulphuric acid. About 190 g. of light gray precipitate were collected....."Fraction V".

### Fraction. I.

The silver precipitate (116 g. from Lot. 1,.....142 g. from Lot. 2.) obtained at  $P_H$  2.5, was suspended in water, and decomposed by hydrochloric acid.

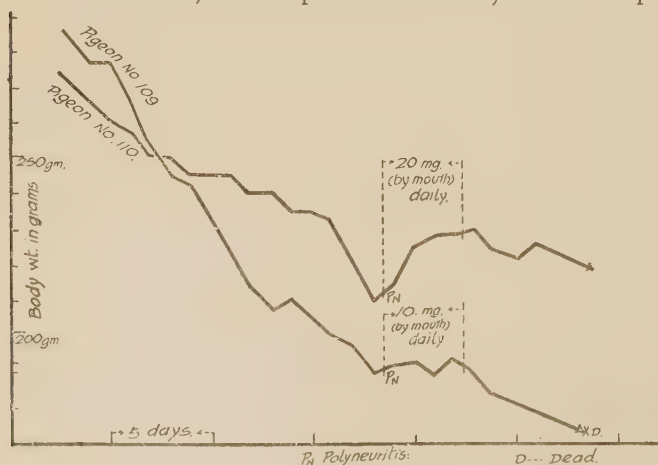


Chart. 8—Pigeons on polished rice, Fraction I.

water. The filtrate from barium phosphotungstate, freed from an excess of

The light brown filtrate was treated with a requisite quantity of sulphuric acid to make 5% acidity and then with a 50% aqueous solution of phosphotungstic acid. The precipitate was filtered by suction after standing 24 hours, dissolved in diluted acetone and decomposed by the addition of baryta

bayta with sulphuric acid, were concentrated to a small volume. These each contained 21.7 g. (Lot. 1) and 22.5 g. (Lot. 2) of total solids which showed a negligible activity for pigeons. (Chart 8).

On further concentration, white micro-crystals were separated out.

Yield: 32 g. from 1 kg. of active oryzanin.

(1) *Adenin*  $C_5H_5N_5$ :— Recrystallised from hot water yield: 25 g. in pure state. White short needles, sparingly soluble in water, gives Kossel's adenin reaction. Its picrate crystallised in characteristic long yellow needles, sparingly soluble in water.

No.	Subst mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C%	H%	N%
(1)	4.211	6.843	1.416	44.35	3.74	—
(2)	4.838	7.855	1.826	44.28	4.17	—
(3)	1.814	0.802 c.c. N (15°C 757 mm.)			—	52.01
(4)	2.248	0.982 c.c. N (17°C 760 mm.)			—	51.63
(5)	1.902	0.836 c.c. N (16°C 759 mm.)			—	51.87
Calc. for $C_5H_5N_5$				44.44	3.71	51.85

Analysis of the picrate:

No.	Subst mg.	Vol. of N, cc	Temp. C.	Press. mm	N%
(1)	3.257	0.835	15	759	30.37
(2)	3.253	0.845	16	759	30.65
Calc. for $C_5H_5N_5-C_6H_3N_3O_7$					30.79

(2) *Adenin-Hypoxanthin*:— The filtrate from adenin, gave on evaporation, 9 g. of fine crystals which were recrystallised from hot water. Yield: 5.3 g. White needles, decomposed at 320°C (uncorr.). It gives Kossel's adenin reaction while Weidel's, xanthin and diazo-reactions are all negative, however; unlike adenin it is easier soluble in water.

No.	Subst mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C%	H%	N%
(1)	7.040	11.434	2.417	44.29	3.81	—
(2)	2.216	0.862 c.c. N (14°C 764 mm.)			—	46.55
(3)	2.010	0.857 c.c. N (15°C 766 mm.)			—	46.46
Calc. for $C_5H_5N_5-C_5H_4N_4O$				44.28	3.32	46.50

For the purpose of separating adenin from hypoxanthin, it was converted into picrate and subjected to fractional crystallisation, whereby adenin picrate separated out first from the aqueous solution, forming a long yellow needles.

## Adenin picrate :

No.	Subst mg.	Vol. of N. cc	Temp. C	Press. mm.	N%
(1)	3.144	0.818	17°	764	30.81
(2)	3.030	0.784	17°	764	30.63
Calc. for $C_5H_5N_5-C_6H_3N_3O_7$					30.79

Hypoxanthin picrate: The filtrate of adenin picrate when evaporated to a small volume, separated out the crystals of hypoxanthin picrate forming light yellow plates, melting at 245°C. (uncorr.).

No.	Subst mg.	Vol. of N. cc	Temp. C	Press. mm.	N%
(1)	3.108	0.706	17°	758	26.66
Calc. for $C_5H_4N_4O-C_6H_3N_3O_7$					26.85

(3) *Adenin picrate*:— The filtrate of adenin hypoxanthin was evaporated to a small volume and added with picric acid. Upon standing, adenin picrate separated out which was recrystallised from hot water. Yield 1.5 g. Long yellow needles, sparingly soluble in water.

No.	Subst mg.	Vol. of N. cc	Temp. C	Press. mm.	N%
(1)	1.588	0.414	18.5	760	30.54
(2)	1.764	0.438	20.5	760	30.61
Calc. for $C_5H_5N_5-C_6H_3N_3O_7$					30.79

(4) *Hypoxanthin picrate*:— The filtrate of adenin picrate when concentrated further, separated out another picrates which were collected and recrystallised from dilute alcohol. Yield: 0.7 g. M. p. 246°C. (uncorr.).

No.	Subst mg.	Vol. of N. cc	Temp. C	Press. mm.	N%
(1)	5.027	1.156	20°	752	26.60
(2)	3.824	0.872	17°	760	26.87
Calc. for $C_5H_4N_4O-C_6H_3N_3O_7$					26.85

The above results agrees fairly with hypoxanthin picrate.

## Fraction II.

The silver precipitate (403 g. from Lot 1,..... 360 g. from Lot 2), obtained at the  $P_H$  2.5~4.5, was suspended in water and decomposed by adding an excess of hydrochloric acid. The filtrate from silver chloride was added with the requisite quantity of sulphuric acid to make the acidity of the solution 5 %  $H_2SO_4$ , and precipitated with phosphotungstic acid. The precipitate dissolved in diluted acetone, was decomposed with baryta water and



the filtrate was concentrated in vacuum after removing the excess of baryta with sulphuric acid. It contained 96.7 g. of total solid but showed to be inactive. (Chart. 9).

The solution, when evaporated further and kept in a cold place, separated out 67.8 g. of nicotinic acid in needles.

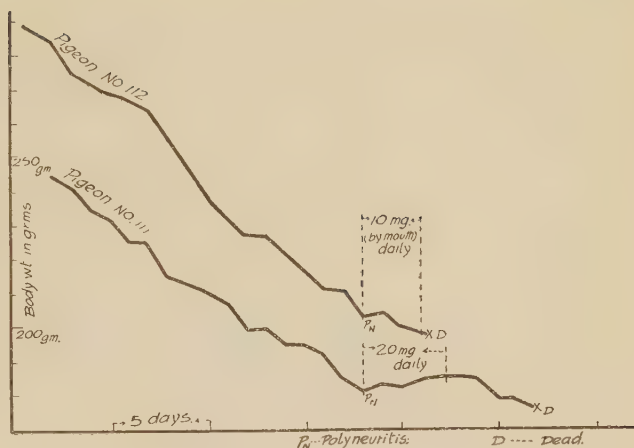


Chart 9—Pigeons on polished rice. Fraction II.

(5) *Nicotinic acid*:— Recrystallised from hot water. Yield: 53.1 g. from 1 kg. of active oryzanin (Lot. 1 & 2). White needles soluble in water and alcohol, melting at 235°C. (uncorr.). Kossel's adenin-reaction as well as Pawly's diazo-reaction were all negative.

No.	Subst. mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C%	H%	N%
1 (Lot No. 1)	4.928	10.275	1.948	58.06	4.39	—
2 "	4.855	10.388	1.893	58.36	4.34	—
3 (Lot No. 2)	4.337	9.019	1.586	58.60	4.06	—
4 "	4.992	10.720	2.011	58.60	4.48	—
5 (Lot No. 1)	5.523	0.5341 c.c. N (15°C 770 mm.)			—	11.01
6 "	3.754	0.346 c.c. N (15°C 772 mm.)			—	11.10
7 "	5.969	0.600 c.c. N (20°C 750 mm.)			—	11.55
8 "	4.183	0.409 c.c. N (20°C 760 mm.)			—	11.38
Calc. for, C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub>				58.54	4.07	11.38

Picrate of nicotinic acid: Light yellow plates, m. p. 219° (uncorr.).

No.	Subst. mg.	Vol. of N, cc	Temp. C	Press. mm.	N%
(1)	3.701	0.510	18°	760	16.16
(2)	3.069	0.416	18°	760	16.09
Calc. for, C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub> -C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub>					15.91

*Adenin picrate*:— The filtrate from the above crystals when added with picric acid, separated out adenin picrat which were filtered and recrystallised from hot water. Yield 1.3 g.

No.	Subst. mg.	Vol. of N, cc	Temp. C	Press. mm.	N%
(1)	2.625	0.689	19°	763	30.76
(2)	2.119	0.548	18°	763	30.48
Calc. for C <sub>5</sub> H <sub>5</sub> N <sub>5</sub> -C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub>					30.79

(8) *Picrate of nicotinic acid*:— The filtrate of the above picrate, by concentrating further, separated out another picrate which were collected and recrystallised from hot dilute alcohol. Yield: 21.3 g. Light yellow plates, m. p. 219°C (uncorr.).

No.	Subst. mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C%	H%	N%
(1) Lot No. 1	5.792	8.724	1.143	41.08	2.19	—
(2) Lot No. 2	5.607	8.383	1.137	40.78	2.25	—
(3) Lot No. 1	5.526	0.792 c.c. N (14°C 767 mm.)			—	15.77
(4) Lot No. 1	4.503	0.593 c.c. N (15°C 768 mm.)			—	15.77
(5) Lot No. 2	4.166	0.550 c.c. N (15°C 761 mm.)			—	15.68
(6) Lot No. 2	4.439	0.594 c.c. N (15°C 762 mm.)			—	15.59
Calc. for C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub> -C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub>				41.19	2.27	15.91

### Fraction III.

The silver precipitate obtained at the P<sub>H</sub> 6.8 was decomposed by triturating with an excess of hydrochloric acid. On filtration, a clear light brown solution was obtained which was diluted to 2 litres. The acidity of the solution having been measured by titration, enough sulphuric acid was added to make it 5% acidity and then a 50% aqueous solution of phosphotungstic acid was added. Usually about 50 g. of phosphotungstic acid were required. The precipitate, after standing for 24 hours, was filtered by suction, washed with 5% sulphuric acid, dissolved in diluted acetone and filtered from a very small amount of undissolved residue. The clear dark brown filtrate was poured gradually into about 2 litres of 5% sulphuric acid with constant stirring. A voluminous precipitate, produced thereby, after standing over night, was filtered by suction and collected on a porous plate. Yield: about 50 g. in average. The dark gray precipitate was now dissolved in dilute acetone and decomposed by adding gradually with the saturated solution of baryta until the solution reacted very slightly alkaline. The precipitate of barium phosphotungstate was filtered off by suction immediately. After acidifying it with sulphuric acid quickly and filtering from barium sulphate, the filtrate was concentrated in vacuum to remove acetone. Further the solution freed from an excess of sulphuric acid with barium chloride exactly, was concentrated to a small volume and then added with alcohol. On standing in refrigerator for a day, whereby separated out crystals which were filtered. Crude product: 9.8 g. from 2 kg. of active Oryzanin.

(8) *Hydrochloride of nicotinic acid*:— Recrystallised from dilute alcohol. Yield: 2 g. Colorless plates, readily soluble in water, sparingly in alcohol and insoluble in acetone, benzene etc. m. p. 267~268°C. (uncorr.). Kossel's and diazo-reactions were all negative. The test on pigeons showed that it was entirely inactive. (Chart. 10).

No.	Subst mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C%	H%	N%	N%
(1)	5.668	9.497	2.038	45.69	3.99	—	—
(2)	5.107	8.464	1.799	45.25	3.92	—	—
(3)	4.452	7.204	1.520	45.14	3.89	—	—
(4)	5.842	0.4312 c.c. N (15.5°C 760 mm.)			—	8.72	—
(5)	5.116	0.3822 c.c. N (21.0°C 751 mm.)			—	8.75	—
(6)	7.102	6.058 AgCl		—	—	—	21.34
(7)	6.251	5.403 AgCl		—	—	—	21.63
Calc. for C <sub>5</sub> H <sub>4</sub> NCO <sub>2</sub> H-HCl				45.28	3.81	8.81	22.10

Picrate of nicotinic acid: Its picrate forms light yellow plates, melting at 219°C. (uncorr.).

No.	Subst mg.	Vol. of N. c.c.	Temp. C	Press. mm.	N%
(1)	4.142	0.589	20°	767	15.97
(2)	4.209	0.577	20°	752	15.80
Calc. for C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub> -C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub>					15.91

The alcoholic filtrate from nicotinic acid was concentrated further and finally dried in vacuum over soda-lime. The resulting product was a light brown colored hygroscopic mass which cured pigeons from polynueritis in the doses of 0.5 mg. daily. (Chart. 11). Yield: 5 g. from 100g. of active oryzanin. (Lot 1 ..... 22.1 g., Lot 2 ..... 24.6 g., Lot 3 ..... 20.2 g., Lot 4 ..... 27.0 g.).

From its activity, this product contains 10,000 units for pigeons, i.e., the yield corresponds to about 40% of the total activity existed in active oryzanin or 13% of the same in the original rice-polishings.

The product was now treated with absolute alcohol, only a small part remaining undissolved. The light brownish colored extract, after filtration, was treated with a 5% solution of platinum chloride in absolute alcohol. About 2~3 g. of platinum chloride were required usually. The orange yellow precipitate formed thus, filtered by suction after standing over night and washed with absolute alcohol.

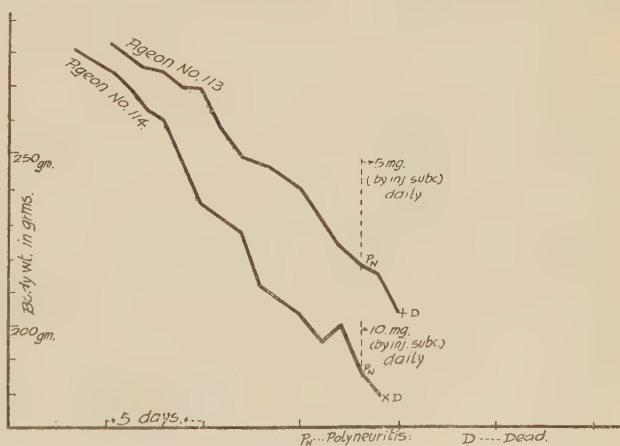
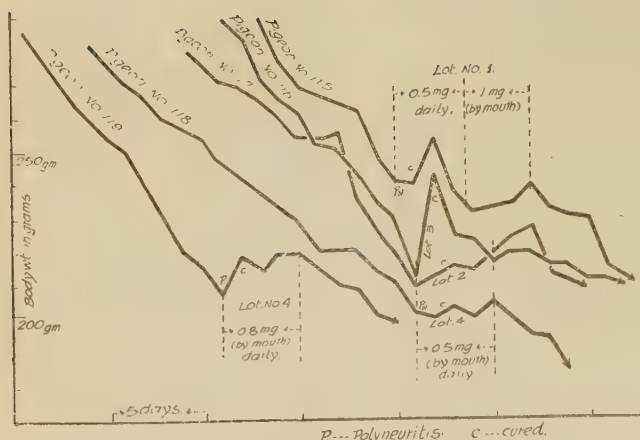


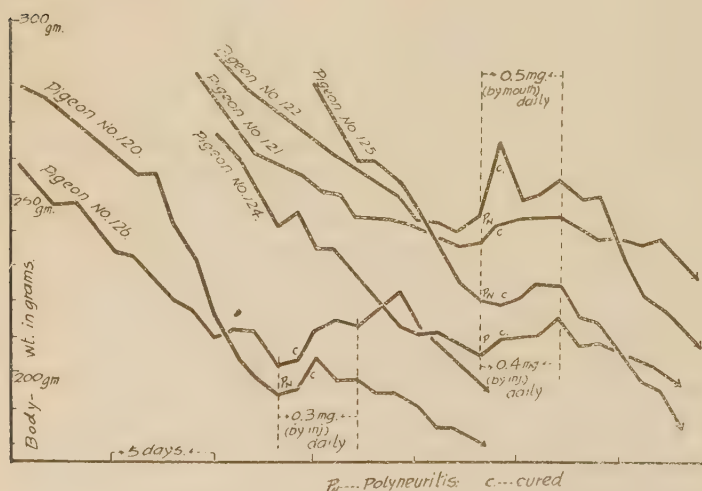
Chart. 10—Pigeons on polished rice  
(8) Hydrochloride of Nicotinic acid.





Chart, 11—Pigeons on polished rice  
Fraction III, ppt-phosphotungstic acid,

pigeons in daily doses of 0.3~0.5 mg. (Chart. 12). Yield: 2 g. in average, (Lot 1.....10.53 g, Lot 2.....10.4 g, Lot 3.....8.06 g, Lot. 4...11.82 g.) i.e.



Chart, 12—Pigeons on polished rice, Fraction III, ppt-PtCl<sub>3</sub>.

i) The platinum precipitate, after being suspended in about 50 c.c. of water acidulated with few drops of hydrochloric acid, was decomposed with sulphureted hydrogen gas and filtered. The clear filtrate was evaporated to a syrupy consistency and dried in vacuum over soda-lime. The resulting hygroscopic light brown mass was active for

corresponded to about 25% of the active substance contained in active oryzanin or to about 8.8% of that in original rice-polishings.

The product was dissolved in a small volume of absolute alcohol and filtered from a small amount of insoluble substance.

For the purpose of

the fractional precipitation, acetone was added cautiously to it until a white turbidity was caused. When settled over night, it subsided to the bottom forming a dark brown resinous substance which was discarded being inactive for pigeons. The solution decanted, when added with a small volume of acetone again, kept in a cool-place, separated out crystalline spherules contaminated partly with resinous substance. The supernatant fluid was decanted and spherules were collected by dissolving the resinous substance with a small

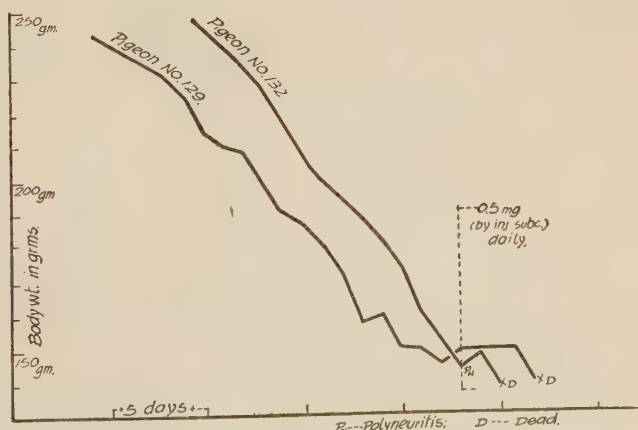
volume of absolute alcohol whereby spherules only remained undissolved. The yield of the crude product was 2.2 g. from 2 kg. of active oryzanin. (Lot 1...0.3 g., Lot 2...1.02 g., Lot 3...0.3 g., Lot 4...0.1 g.).

(9) *Unknown base hydrochloride*.  $(C_3H_6N_2-HCl)_n$ :— Recrystallised from dilute alcohol. Yield: 1.7 g. It forms colorless thick plates, readily soluble in water, sparingly in absolute alcohol, melting at  $267^\circ C$  (uncorr.) with decomposition. Diazo-, biuret-, and purin- reactions are all negative.

No.	Subst mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	AgCl mg.	C %	H %	N %	Cl %
(1) Lot No. 1	4.767	5.829	1.897	—	33.35	4.42	—	—
(2) "	6.419	7.961	3.133	—	33.82	5.42	—	—
(3) "	4.715	0.985 c.c. N (16°C 769 mm.)	—	—	—	—	24.97	—
(4) "	3.276	0.676 c.c. N (16°C 771 mm.)	—	—	—	—	24.93	—
(5) "	4.975	1.020 c.c. N (16°C 761 mm.)	—	—	—	—	24.34	—
(6) "	6.288	—	—	7.766	—	—	—	31.19
(7) "	6.161	—	—	7.723	—	—	—	31.35
(8) Lot No. 2	5.969	7.693	3.366	—	35.15	6.26	—	—
(9) "	4.239	5.455	2.350	—	35.09	6.16	—	—
(10) "	5.265	6.609	2.941	—	34.23	6.21	—	—
(11) "	5.271	6.620	2.880	—	34.08	6.07	—	—
(12) "	1.988	0.354 c.c. N (17°C 767 mm.)	—	—	—	—	21.15	—
(13) "	4.102	0.747 c.c. N (18°C 767 mm.)	—	—	—	—	21.57	—
(14) "	2.935	0.531 c.c. N (18°C 767 mm.)	—	—	—	—	21.43	—
(15) "	6.361	—	—	8.376	—	—	—	32.92
(16) Lot No. 3	4.332	5.527	2.366	—	34.79	6.07	—	—
(17) "	5.223	6.653	2.863	—	34.73	6.09	—	—
(18) "	4.721	6.010	2.555	—	34.72	6.02	—	—
(19) "	4.105	0.861 c.c. N (17°C 767 mm.)	—	—	—	—	24.93	—
(20) "	4.125	0.863 c.c. N (15°C 761 mm.)	—	—	—	—	24.86	—
Calc. for $C_3H_6N_2-HCl$					33.96	6.60	26.42	33.33

From these results, it is clear that the compound is identical with the base<sup>(4)</sup>  $(C_3H_6N_2)_n$  isolated from yeast extract by the present author. This compound was entirely inactive for pigeons. (Chart. 13.).

The acetone solution, decanted from the above, was treated further with acetone



Chart, 13—Pigeons on polished-rice.  
Hydrochloride of unknown base  $(C_3H_6N_2-HCl)_n$ .

until a white turbidity is produced and was placed in a refrigerator. These treatment was repeated day by day. After a week, crystalline spherules contaminated partly with a resinous substance separated out to the bottom and the wall of the vessel. The supernatant fluid was now decanted into another vessel and treated with more acetone, whereby a second crystallization was obtained. The crude crystals adhering to the vessel could be collected easily by treating with a small volume of absolute alcohol, the resinous substance being easily soluble in the latter. The crude crystals: 0.1 g. commonly. By treating 2 kg. of active oryzanin in this way, about 1.3 g. were obtained.

*Oryzanin hydrochloride*:— The crude product was now dissolved in dilute alcohol, decolorised with a small quantity of charcoal, and recrystallised by adding absolute alcohol and acetone. Yield: 0.05 g. commonly from 100 g. of active oryzanin.

It crystallises in colorless, monoclinic long plates, readily soluble in water, sparingly in absolute alcohol, but insoluble in acetone, benzene and ether etc. It gives yellowish coloration instantly which turns to red gradually when added with Pauly's diazo-reagent and gives a white precipitate by phosphotungstic acid, also by mercuric sulphate.

It contains sulphur in the form, detectable by sodium nitro-prusside or by lead acetate when it is previously boiled with alkali or fused with metallic sodium. From its melting point as well as from its activity for pigeons, it was ascertained, however, that a slight difference existed in each preparations, namely the crystals melting at 253°C (uncorr.) were only slightly active, while those melting at 220°C (uncorr.) were active. Nevertheless, it was revealed by the polarization microscope that the crystals were still contaminated with some amorphous substance. Only the crystals melting at 250°C (uncorr.) were quite uniform and possessed the highest activity.

Total yield 0.85 g. from 2 kg. of active oryzanin.

Lot No.	Crude product g.	Crystals, g.	Melting Point, C.	Activity for Pigeons, tested mg.	Chart No.
1 {A	0.10	0.05	237°	0.01~0.05	31
B	0.15	0.10	253°	0.01~0.05	32
2 {A	0.05	0.03	250°	0.01	33
B	0.23	0.19	221°	0.01~0.025	34
3 {A	0.20	0.11	244°	0.01~0.02	35
B	0.58	0.37	220°	0.01~0.04	36

Further study on this compound is described under "Properties and analysis of oryzanin hydrochloride".

The supernatant fluid decanted from the above crystals, when added with acetone more, kept in a refrigerator, separated out occasionally crystalline spherules which were collected after standing for a week. Yield 0.7 g. from



2 kg. of active oryzanin.

(11) *Unknown base hydrochloride*.  $(C_3H_6N_2-HCl)_n$ :— Recrystallised from dilute alcohol. Yield: 0.37 g. (Lot 2...0.11 g., Lot 3...0.1 g., Lot 4...0.12 g.) Colorless plates; melting at  $266^\circ C$ . (uncorr.).

No.	Subst mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C %	H %	N %
(1)	4.928	6.254	2.741	34.62	6.18	—
(2)	5.302	6.775	2.931	34.85	6.15	—
(3)	4.102	0.85 c.c. N ( $19^\circ C$ 760 mm.)			—	24.24
(4)	4.018	0.82 c.c. N ( $19^\circ C$ 760 mm.)			—	23.93
Calc. for, $C_3H_6N_2-HCl$				33.96	6.60	26.42

From its property and analytical result, it was assumed, this compound is identical with that described under (No. 9). The biological test showed it to be entirely inactive for pigeons. (Chart 14).

B) When the alcoholic solution filtered from (9), (10) and (11) was treated with acetone again, and a light yellow hygroscopic substance obtained, which is readily soluble in alcohol and water but insoluble in acetone. It gives intensive diazoreaction. Yield: 2 g. from 500 g. of active oryzanin (Lot. 1) Pigeon-test showed it to be of negligible activity (Chart. 15).

C) The acetone

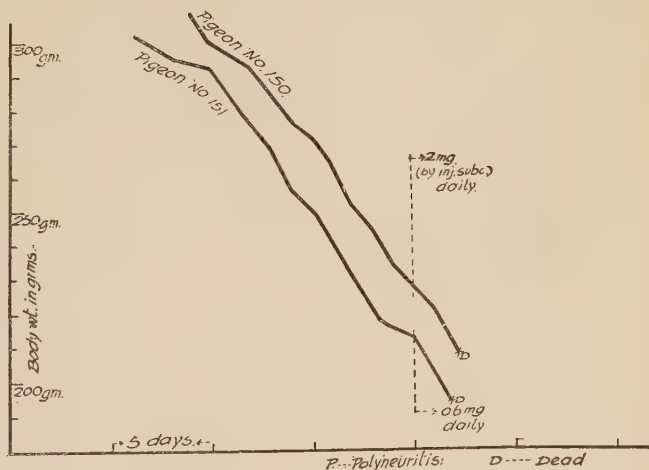


Chart. 14—Pigeons on polished rice.  
(11) Hydrochloride of Unknown base,  $C_3H_6N_2-HCl$

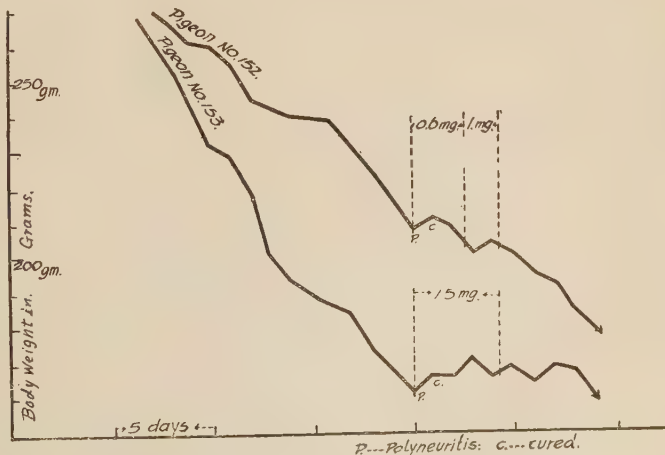


Chart. 15—Pigeons on polished rice.  
Amorphous substance, insol in acetone.

solution decanted from the above (A) and (B) was evaporated. The residue was dissolved again in small quantity of absolute alcohol, and filtered from a small amount of insoluble substance. By keeping it in a refrigerator for a week, hydrochloride of nicotinic acid crystallised out. Yield: 0.45 g. from 1 kg. of active oryzanin. (Lot 1. & 2.).

(12) *Hydrochloride of nicotinic acid*:— Recrystallised repeatedly from dilute alcohol. Yield: 0.17 g. Colorless plates, m. p. 266°C (uncorr.). Biological test resulted to be entirely inactive for pigeons. (Chart. 16.).

No.	Subst. mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C %	H %	N %
(1)	4.874	8.114	1.752	45.41	3.99	—
(2)	4.278	7.121	1.472	45.39	3.83	—
(3)	3.942	0.292 c.c. N (18°C 760 mm.)				8.69
(4)	2.705	0.197 c.c. N (18°C 760 mm.)				8.42
Calc. for C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub> -HCl				45.28	3.81	8.81

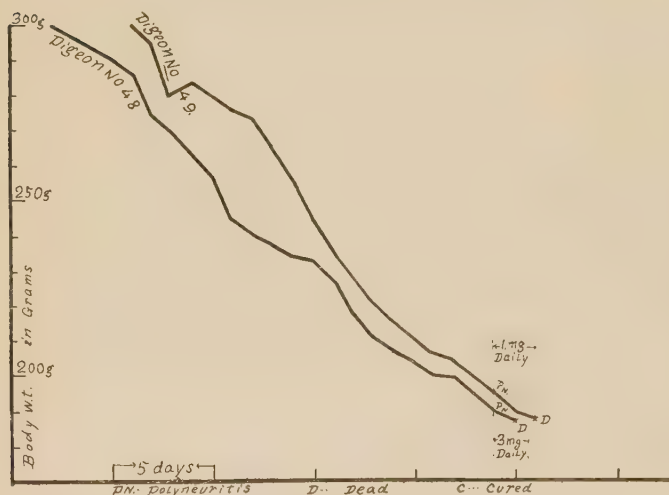
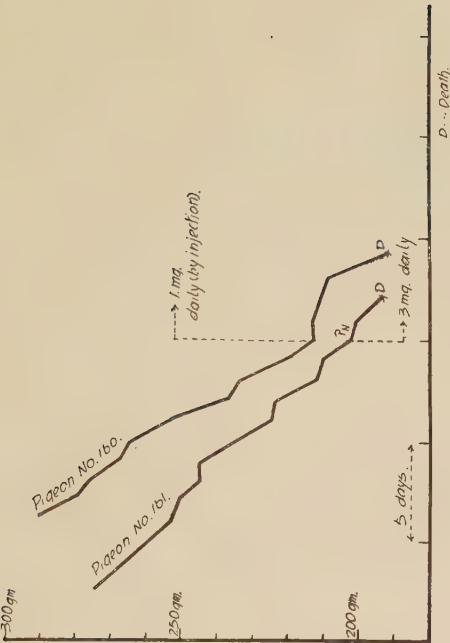


Chart. 16—Pigeons on Polished rice  
(12) Hydrochloride of Nicotinic acid.

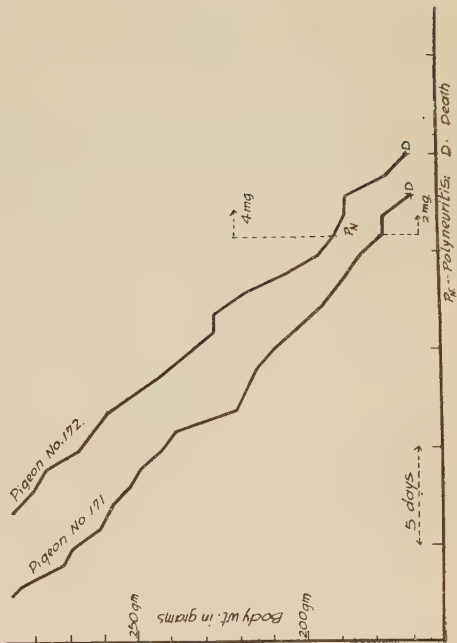
The alcoholic filtrate of the above (12), which separated no more crystals by treating with acetone, was evaporated in vacuum to dryness, and about 0.8 g. of a light brown amorphous substance were obtained from 100 g. of active oryzanin. (Lot 1...4.12 g., Lot 2. & 3...7.51g.). It is readily soluble in water and alcohol, It gives an intensive

• diazoreaction, but it was almost inactive for pigeons. (Chart. 17.).

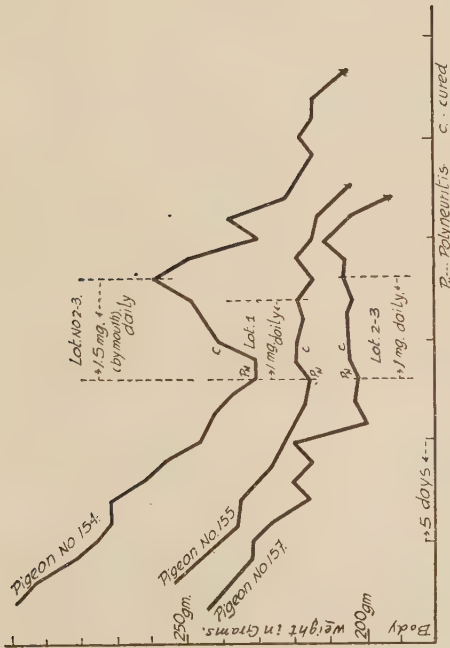
ii) The alcoholic solution filtered from the platinum precipitate (i) was evaporated and the residue was treated with sulphuretted hydrogen gas after suspended in water, whereby much quantity of the insoluble resinous substance separated out together with platinum sulphide which were filtered off. The filtrate was concentrated in vacuum to dryness. About 5.3 g. of a brown amorphous substance were thus obtained from Lot. 1. The product being inactive for pigeons (Chart. 18) it was proved that all active substance had been precipitated by the above platinum treatment.



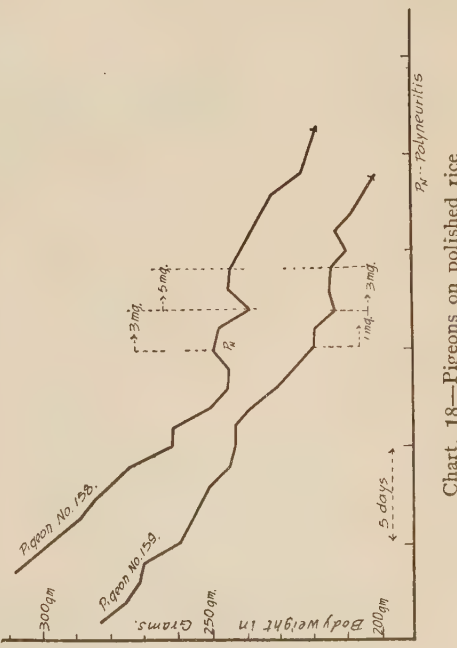
Chart, 19—Pigeons on polished rice  
(13) Hydrochloride of nicotinic acid.



Chart, 20—pigeons on polished rice.  
Amorphous substance, Filt-PtCl<sub>4</sub> Sol. in acetone.



Chart, 17—Pigeons on polished rice  
Amorphous substance (sol. in acetone).



Chart, 18—Pigeons on polished rice  
Amorphous substance, Filt-PtCl<sub>4</sub>.



A) The product, dissolved in a small volume of absolute alcohol, treated with acetone in the same manner as the above platinum precipitate. 1.31 g. of the crude hydrochloride of nicotinic acid were obtained. (Lot 1...0.56 g., Lot 2...0.75 g.).

(13) *Hydrochloride of nicotinic acid*: — Recrystallised from dilute alcohol. Yield: 0.75 g. Colorless plates, melting at 267°C. (uncorr.). Biological test showed it to be inactive for pigeons. (Chart, 19).

No.	Subst mg.	Vol. of N. c.c.	Temp. C	Press. mm.	N %
(1)	4.010	0.311	16°	761	9.18
(2)	3.053	0.256	18°	761	9.86
(3)	6.246	0.531	20°	761	9.91
Calc. for $C_6H_5NO_2 \cdot HCl$					8.81

B) The actone solution from the above, was evaporated in vacuum to dryness and a brown amorphous substance were obtained. Yield: 3.25g. from 500 g. of active oryzanin. (Lot. 1). Although it gave strong Pauly's diazoreaction, the product was inactive for pigeons. (Chart. 20).

#### Fraction IV.

The silver precipitate obtained at the  $P_H$  6.8~9.0, was decomposed by hydrochloric acid and precipitated by phosphotungstic acid in the same manner with the preceding fraction. The phosphotungstic precipitate amounted to about 55 g. from 100 g. of active oryzanin.

The filtrate which was obtained by treating the phosphotungstic precipitate with baryta, was concentrated to a small quantity and dried in vacuum over soda-lime. Thus, 42.7g. of a light brown mass were obtained by treating

500 g. of active oryzanin (Lot. 1). i.e. approximately 8.5 % of active oryzanin. This product, being active in doses of 5 mg. for pigeons (Chart. 21) contains still about 6.8% of the activity existed originally in active oryzanin or about 2.3 % of that contained



Chart. 21—Pigeons on polished rice, Fraction VI.

in original rice polishings. The product was then treated with absolute al-

cohol, which dissolved the greater part and a brown substance undissolved was filtered off. The alcoholic solution was now added with a 5% solution of platinum chloride in absolute alcohol and the precipitate thus formed, was filtered by suction after standing over night in a refrigerator.

(i) The platinum precipitate which was suspended in water, decomposed by sulphuretted hydrogen gas and filtered from platinum sulphide after standing over night. The filtrate was evaporated to a small quantity and dried in vacuum over soda-lime. The resulting light brown mass weighed 12 g. from 500 g. of active oryzanin (Lot 1), and its curative daily dose was 1~2 mg. for a pigeon. (Chart 22).

The product dissolved in absolute alcohol, filtered from undissolved substance and acetone was added cautiously to it in the same manner with fraction III. The solution decanted from a dark brown substance,

separated out crystalline spherules when kept it in a refrigerator. The supernatant fluid was decanted and these spherules were collected by treating with small quantity of absolute alcohol. Weighed: 0.35 g. (Lot. 1).

(14) *Unknown base Hydrochloride*. ( $C_3H_6N_2-HCl$ )<sub>n</sub>: — Recrystallised repeatedly from dilute alcohol, it forms colorless long plates, melting at 262°C. (uncorr.) with decomposition and diazo-reaction was negative. Yield: 0.2g. (from 500 g. of active oryzanin). It was inactive for pigeons. (Chart. 23).

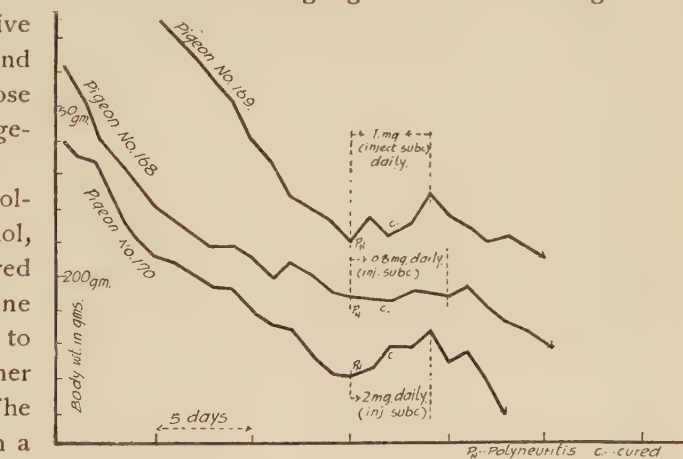


Chart. 22—Pigeons on polished rice, Fraction IV, PPT- $PtCl_4$ .

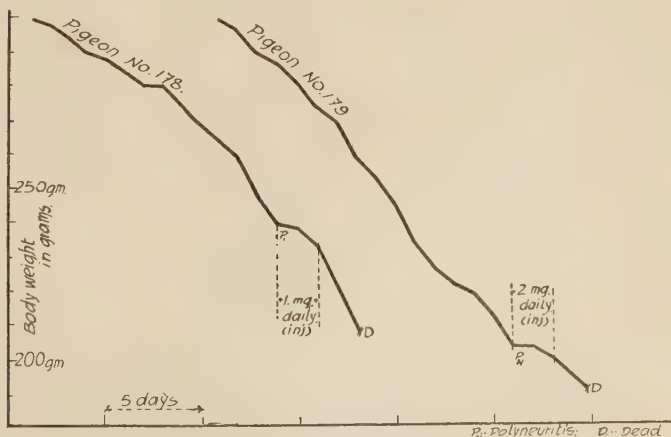


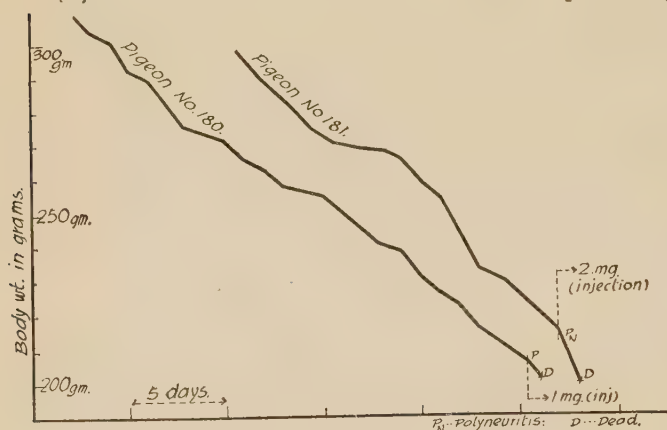
Chart. 23—Pigeons on polished rice,

(14) Hydrochloride of unknown-base ( $C_3H_6N_2-HCl$ )

No.	Subst. mg.	Vol. of N. c.c.	Temp. C	Press. mm.	N %
(1)	4.050	0.845	18°	763	24.57
(2)	4.711	1.000	17°	763	25.08
(3)	4.317	0.894	11°	756	24.81
(4)	5.838	1.235	12°	756	25.21
Calc. for $C_3H_6N_2-HCl$					26.42

From its properties and analytical results, it was assumed to be identical with the hydrochloride (9).

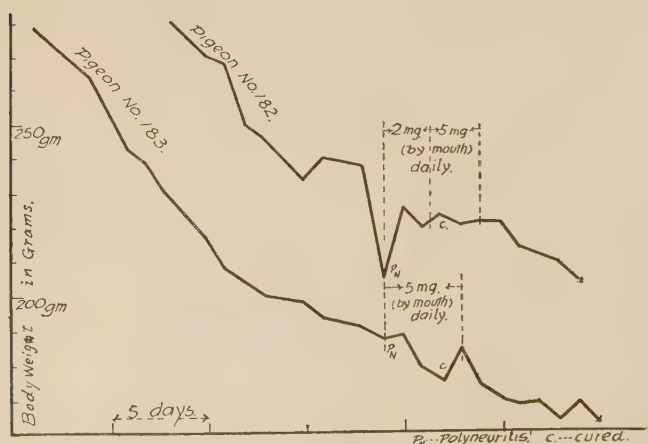
(B) The alcoholic solution filtered from preceding crystals, was treated



Chart, 24—Pigeons on polished rice.

Amorphous substance, Fraction VI, Sol. in acetone.

tone solution decanted from the above fractions (A) and (B), giving no more



Chart, 25—Pigeon on polished rice.

Amorphous substance, Fraction VI, sol. in acetone.

showed it to be slightly active even in daily doses of 5 mg. (Chart. 25).

again with acetone. The resulting hygroscopic amorphous substance, readily soluble in water and alcohol, gave intensive diazo-reaction, nevertheless it was inactive for pigeons. (Chart. 24.). Yield: 5.6 g. from 500 g. of active oryzanin (Lot 1.)

(C) The acetone solution decanted from the above fractions (A) and (B), giving no more crystalline substance by repeating the same treatment, was therefore concentrated in vacuum and dried over soda-lime. 5.6 g. of a light brown amorphous substance were obtained from 50 g. of active oryzanin, (Lot 1). It is readily soluble in water, alcohol, and gives strong diazo-reaction. Pigeon curative test



(ii) The alcoholic solution filtered from platinum precipitate, was evaporated and the residue suspended in water, was decomposed by sulphuretted hydrogen gas. The filtrate was evaporated in vacuum and dried over soda-lime. The resulting mass, was extracted with absolute alcohol and filtered.

By adding with acetone gradually, it separated out an amorphous substance at first and then crystalline spherules when kept in a refrigerator. The supernatant fluid decanted off and the spherules were collected by treating with absolute alcohol in the same manner with previous fraction. Yield: 1.5 g. (Lot 1.).

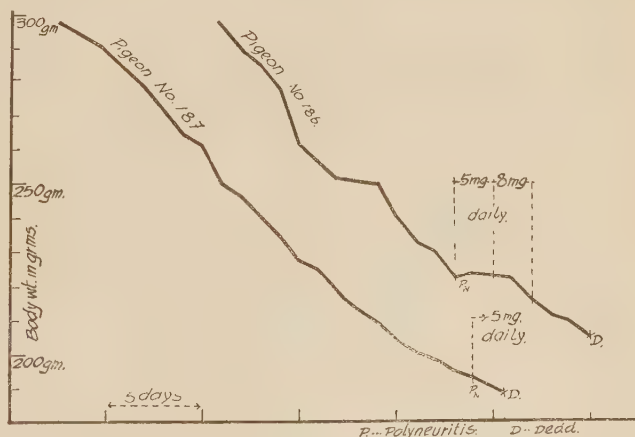
(15) *Hydrochloride of nicotinic acid*: — Those crystals, still mixed with inorganic substance, recrystallised repeatedly from hot alcohol. Yield: 0.3 g. (from 500 g. of active oryzanin. Lot. 1). Colorless plates, melting at 266 °C (uncorr.). It was inactive for pigeons. (Chart. 26).

(B) The acetone solution decanted from the above which contains still solids, was evaporated and dried



Chart, 26—Pigeons on polished rice.

(15) Hydrochloride of Nicotinic acid.



Chart, 27—Pigeons on polished rice.

Amorphous substance, Fraction IV, Filt-PtCl<sub>4</sub>.

in vacuum over soda-lime. 2.5 g. of a light brown amorphous substance were obtained from 500 g. of active oryzanin. It gives strong diazo-reaction but pigeon curative test showed it to be inactive. (Chart. 27.).

No.	Subst. mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C %	H%	N%
(1)	5.175	7.363	1.830	39.81	3.93	—
(2)	5.216	7.819	1.798	39.14	3.84	—
(3)	5.780	0.4067 c.c. N (16°C 761 mm.)			—	8.32
(4)	4.927	0.3528 c.c. N (15°C 762 mm.)			—	8.47
Calc. for, C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub> -HCl				45.28	3.81	8.81

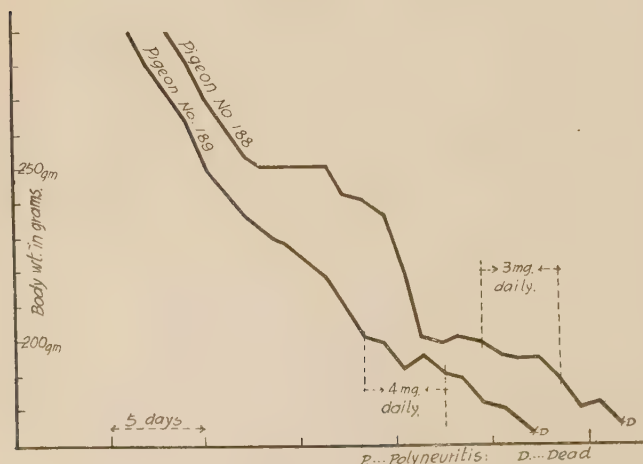
### Fraction V.

The phosphotungstic precipitate (923 g. from Lot 1.....1050 g. from Lot 2.) was dissolved in diluted acetone and decomposed by adding baryta water in slight excess. The basic solution filtered from barium phosphotungstate,

was freed from an excess of baryta with sulphuric acid. After acidifying with hydrochloric acid, it was concentrated in vacuum to a small volume. it contained 232 g. of solids<sup>10</sup> (116 g. from Lot 1.....



Chart, 28—Pigeons on polished rice, Fraction V.



Chart, 29—Pigeons on polished rice,  
(16) Hydrochloride of Nicotinic acid.

115.7 g. from Lot 2) which showed, however, to be almost inactive for pigeons. (Chart. 28.).

The light brownish colored solution was concentrated further to a syrupy consistence and dissolved in alcohol. On keeping the alcoholic solution in a refrigerator, a quantity of crystals separated out. The alcoholic filtrate, also

when concentrated and kept in refrigerator, gave a second crop which was collected. It amounted 44 g. (Lot 1 & 2.).

(16) *Hydrochloride of nicotinic acid*: — Recrystallised repeatedly from hot dilute alcohol. Yield: 34.8 g. (from 1 kg. of active oryzanin.). Colorless plates, readily soluble in water, sparingly in alcohol and insoluble in acetone, benzene etc. It melts at 245°C (uncorr.), and does not give diazoreaction. Pigeon curative test showed quite inactive (Chart. 29.).

No.	Subst. mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C %	H %	N %
(1) Lot No. 1	5.586	9.127	2.088	44.56	4.11	—
(2) "	4.746	7.844	1.696	45.16	3.97	—
(3) "	5.039	0.374 c.c. N (14°C 755 mm.)			—	8.77
(4) "	6.101	0.441 c.c. N (16°C 756 mm.)			—	8.49
(5) Lot No. 2	4.909	8.567	2.219	47.59	5.02	—
(6) "	6.636	8.206	1.946	48.27	4.68	—
(7) "	4.962	8.671	2.079	47.85	4.65	—
(8) "	5.783	0.436 c.c. N (15°C 762 mm.)			—	8.96
(9) "	7.392	0.541 c.c. N (15°C 762 mm.)			—	8.69
Calc. for C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub> -HCl				45.28	3.81	8.81

Picrate of nicotinic acid: The hydrochloride was converted into picrate by adding picric acid to its aqueous solution. It forms light yellow thick plates melting at 219°C. (uncorr.).

No.	Subst. mg.	Vol. of N, c.c.	Temp. C	Press. mm.	N%
(1) Lot No. 1	4.076	0.539	17°	764	15.64
(2) "	5.754	0.595	17°	764	15.62
(3) Lot No. 2	5.462	0.724	17°	762	15.66
(4) "	4.388	0.580	17°	762	15.72
Calc. for C <sub>6</sub> H <sub>5</sub> NO <sub>2</sub> -C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub>					15.91

The alcoholic solution filtered from above crystals was concentrated again and dissolved in absolute alcohol. On keeping the solution in a refrigerator for a week, it gave crystals in plates which were collected.

(17) *Hydrochloride of Unknown base*. (C<sub>8</sub>H<sub>10</sub>NO<sub>3</sub>-HCl): — Recrystallised repeatedly from hot diluted alcohol. Yield: 4.5 g. (from 1 kg. of active oryzanin.).

It forms colorless long plates, readily soluble in water, sparingly in alcohol, insoluble in acetone, benzene, ether etc., melting at 204~205°C.(uncorr.). with decomposition. Its aqueous solution gave strong Pauly's diazo-reaction, but pigeon curative test showed it to be entirely inactive. (Chart. 30).

Its analytical results agreed with the hydrochloride of the compound having the formula C<sub>8</sub>H<sub>10</sub>NO<sub>3</sub>.

Further studies of this compound is reserved.



No.	Subst mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C%	H%	N %	Cl%
(1) Lot No. 1	5.387	9.018	2.276	45.66	4.69	—	—
(2) "	6.108	9.899	2.464	44.20	4.58	—	—
(3) "	4.653	7.770	2.070	45.54	4.94	—	—
(4) "	4.876	8.039	2.129	44.96	4.85	—	—
(5) "	5.432	0.3822 c.c. N (17°C 759 mm.)	—	—	—	8.30	—
(6) "	4.512	0.3283 c.c. N (17°C 760 mm.)	—	—	—	8.06	—
(7) "	5.012	0.3430 c.c. N (17°C 760 mm.)	—	—	—	8.05	—
(8) "	6.047	0.4214 c.c. N (17°C 761 mm.)	—	—	—	8.22	—
(9) Lot No. 2	6.519	11.155	3.242	46.67	5.53	—	—
(10) "	5.140	8.687	2.572	46.09	5.56	—	—
(11) "	5.159	8.783	2.646	46.43	5.69	—	—
(12) "	4.490	7.687	2.238	46.68	5.54	—	—
(13) "	5.050	0.3332 c.c. N (16°C 771 mm.)	—	—	—	7.97	—
(14) "	6.619	0.4381 c.c. N (16°C 770 mm.)	—	—	—	7.92	—
(15) "	6.576	0.4234 c.c. N (14°C 762 mm.)	—	—	—	7.61	—
(16) "	5.765	0.3773 c.c. N (12°C 763 mm.)	—	—	—	7.97	—
(17) "	5.950	3.889 mg. AgCl	—	—	—	—	16.34
(18) "	7.051	4.656 " "	—	—	—	—	16.51
Calc. for C <sub>8</sub> H <sub>10</sub> NO <sub>3</sub> -HCl				47.06	5.39	6.86	17.16

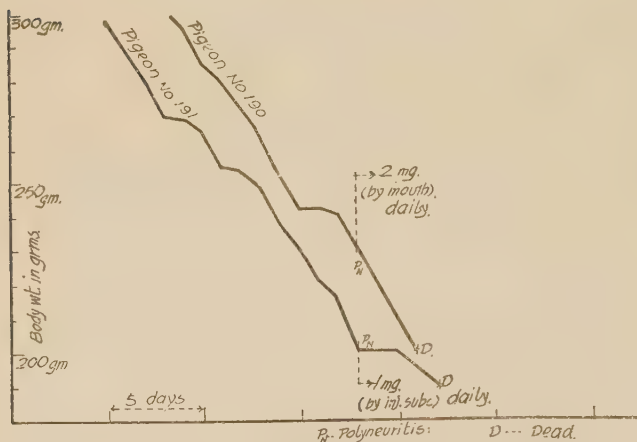


Chart 30—Pigeons on polished rice.  
Hydrochloride of Unknown base C<sub>8</sub>H<sub>10</sub>NO<sub>3</sub>-HCl.

The alcoholic filtrate from the above crystals was evaporated in vacuum. The residue was dissolved in water and filtered from insoluble resinous substance. For the removal of hydrochloric acid in the solution, the requisite quantity of sulphuric acid to make 5% in acidity was added and precipitated again with

phosphotungstic acid. The precipitate, collected by suction after standing over night, dissolved in diluted acetone, was decomposed by adding baryta water in an excess. The alkaline solution, freed from an excess of baryta, was evaporated to a small volume and added with picric acid, whereby cholin picrate separated out in yellow prisms. The filtrate, on further evaporation, separated more crystals of the same picrate. The crude picrate amounted to 13 g. (from 1 kg. of active oryzanin.).

(18) *Cholin picrate*:— Recrystallized from hot dilute alcohol repeatedly. Yield: 10 g. (Lot 1 & 2).

Light yellow prisms, melting at 246°C. (uncorr.).

No.	Subst mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C %	H %	N %
(1)	4.681	6.513	3.051	37.95	5.21	—
(2)	4.754	6.741	2.113	38.67	4.94	—
(3)	5.334	0.7595 c.c. N (22°C 751 mm.)			—	16.26
(4)	5.179	0.730 c.c. N (20°C 751 mm.)			—	16.24
(5)	5.571	0.781 c.c. N (19°C 757 mm.)			—	16.32
Calc. for C <sub>6</sub> H <sub>15</sub> NO <sub>2</sub> ·C <sub>6</sub> H <sub>3</sub> N <sub>3</sub> O <sub>7</sub>				37.71	5.14	16.00

*Properties and analysis of Oryzanin hydrochloride.*

Crystals of Oryzanin hydrochloride, purified further by recrystallisation from alcohol and acetone repeatedly, forms colorless long monoclinic plates (Fig. 1), melting at 250°C (uncorr.) with decomposition and readily soluble in water, sparingly in alcohol, insoluble in acetone, ether, and benzene etc.

Its aqueous solution gives a white precipitate with phosphotungstic acid, or with mercuric sulphate, a dark brown precipitate with iodine potassium iodide and with Dragendorff's reagent, a yellow precipitate with picronic acid but not with picric acid.

It gives sulphur reaction which is detectable by giving a violet coloration with sodium nitroprusside or by giving black precipitates with lead acetate when it is boiled with alkali or fused with metallic sodium previously, while it gives no reaction with these reagents in a aqueous solution itself.

It gives Pauly's diazo-reaction showing quite different coloration to compare with that given by histidine, histamine or by thymin. When it is added with the reagent, it gives yellowish coloration instantly which turn to week redish color gradually after few minutes. It gives also a weak greenish coloration by ferriferricyanide reagent or by phosphomolybdic acid and ammonia while purin-reaction, i.e, Kossel's, Weidel's and xanthin-reactions as well as arginin reaction by  $\alpha$ -naphtol and sodium hypochlorate are all negative.

The alcoholic solution of gold chloride and platinum chloride give its double salts which crystallises in long monoclinic plates.

The crystals, revealed to be uniform under the polarization microscope, melted at 250° and 248,5°C. (uncorr) and were subjected to analysis.

*Analysis of the hydrochloride:—*

No.	Subst mg.	CO <sub>2</sub> mg.	H <sub>2</sub> O mg.	C %	H %	N %	Cl %	S %
[A] M. p. 250.°C								
(1)	4.230	6.286	2.090	40.53	5.49	—	—	—
(2)	4.383	6.455	2.194	40.17	5.56	—	—	—

(3)	2.859	0.379 c.c. N (16°C 755 mm.)				15.55	—	—
(4)	2.734	0.365 c.c. N (15°C 757 mm.)				15.75	—	—
(5)	4.311	3.718 mg. AgCl	—	—	—	21.32	—	—
(6)	5.374	3.614 mg. BaSO <sub>4</sub>	—	—	—	—	9.23	—
[B] M. p. 248,5°C								
(7)	5.412	8.225	2.714	41.44	5.57	—	—	—
(8)	5.235	7.949	2.471	41.41	5.25	—	—	—
(9)	4.385	0.604 c.c. N. (15°C. 757 mm.)				16.26	—	—
(10)	4.915	0.676 c.c. N. (12°C. 752 mm.)				16.29	—	—
(11)	5.499	4.413 mg. AgCl	—	—	—	19.84	—	—
(12)	5.261	4.236 " "	—	—	—	19.91	—	—
(13)	6.031	3.996 mg. BaSO <sub>4</sub>	—	—	—	—	—	9.10
(14)	5.668	3.758 " "	—	—	—	—	—	9.11
Calc. for C <sub>12</sub> H <sub>16</sub> N <sub>4</sub> SO <sub>2</sub> ·2HCl				40.91	5.11	15.91	19.89	9.09
Calc. for C <sub>6</sub> H <sub>10</sub> N <sub>2</sub> O·HCl by Jansen & Donath				44.31	6.77	17.23	21.84	—

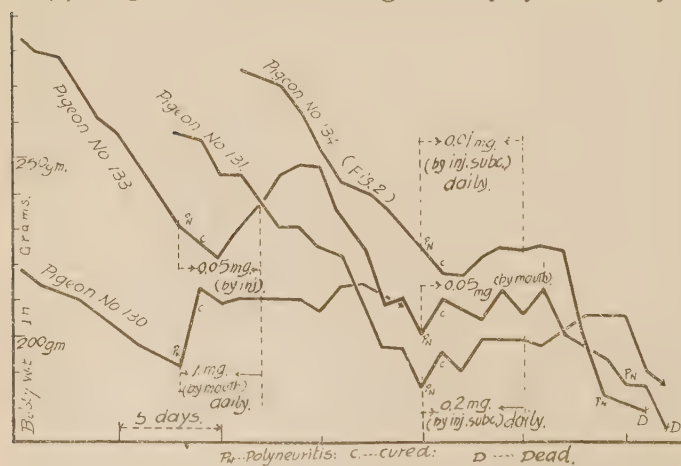
From these results, the antineuric crystals isolated by the author, is apparently the hydrochloride of a new sulphur compound having the empirical formula C<sub>12</sub>H<sub>16</sub>N<sub>4</sub>SO<sub>2</sub> which does not agreed with that of Jansen and Donath unfortunately.

#### Activity of Oryzanin hydrochloride.

The antineuritic activity of the hydrochloride isolated was tested upon both pigeons and white rats with the following results:—

##### [A] Pigeons:

- (1) Pigeons when suffering from polyneuritis by exclusive feeding on polished rice, were injected daily with 0.01 mg. of the crystals before death.



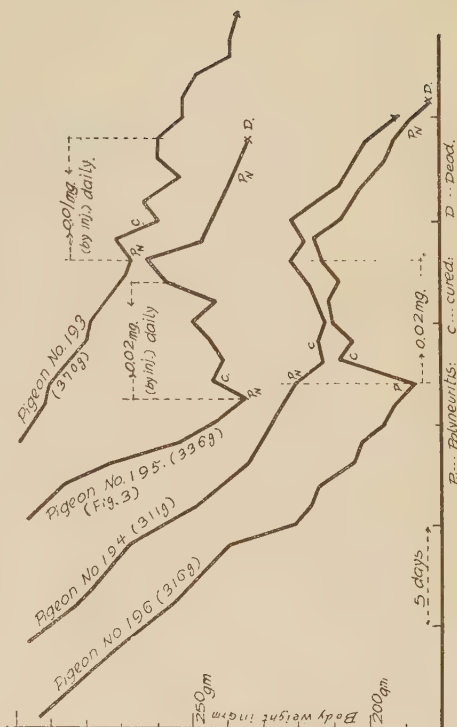
Chart, 31—Pigeons on polished rice,  
Oryzanin hydrochloride, (Lot No. 1, A).

The symptoms were improved in a few hours and cured completely in 1~2 days. (Chart. 31, Fig. II). In daily doses of 0.02 mg. or more, recovery is more rapid and the main symptoms disappeared in 2~3

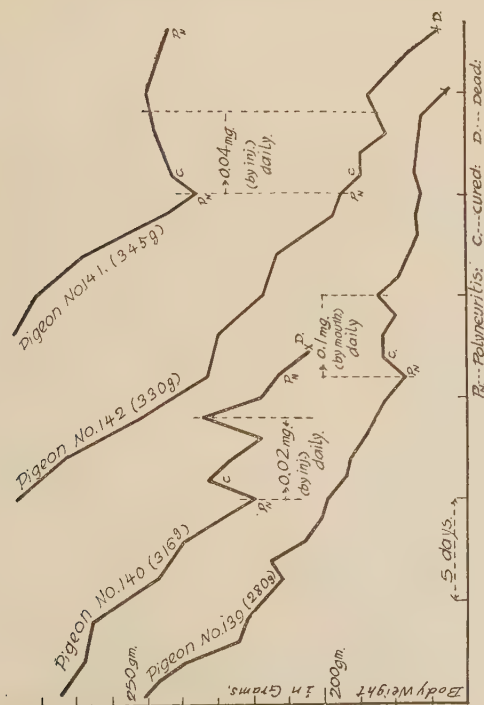




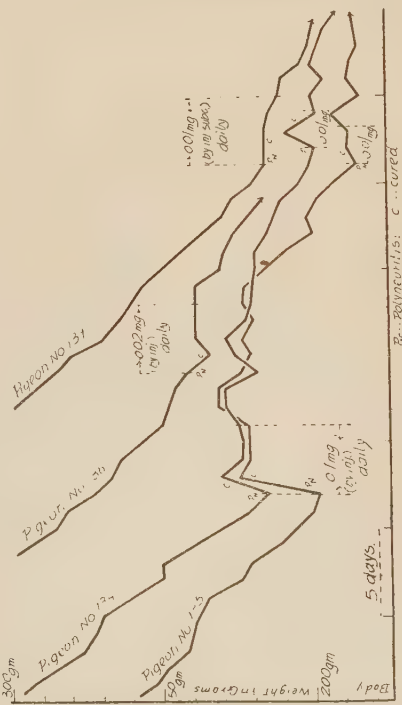
Chart, 34—Pigeons on polished rice, Oryzanin hydrochloride, (Lot No. 3)



Chart, 35—Pigeons on polished rice, Oryzanin hydrochloride, (Lot No. 3, B)



Chart, 32—Pigeons on polished rice, Oryzanin hydrochloride, (Lot No. 1, B)



Chart, 33—Pigeons on polished rice, Oryzanin hydrochloride, (Lot No. 2)

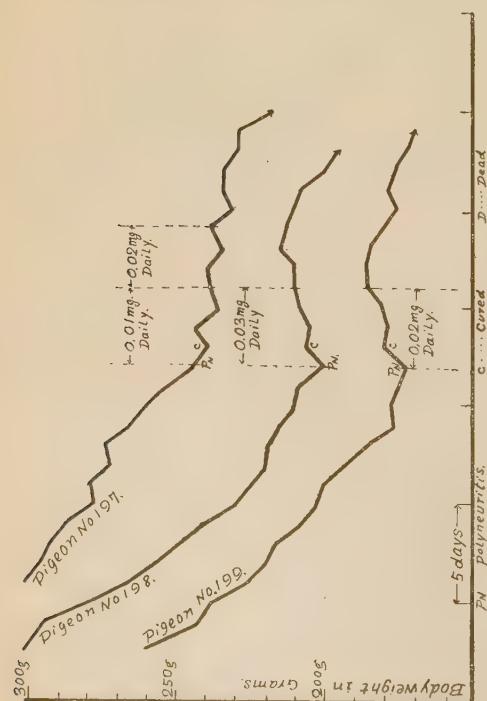


Chart 36—Pigeons on Polished rice,  
Oryzanin hydrochloride (Lot No. 4)

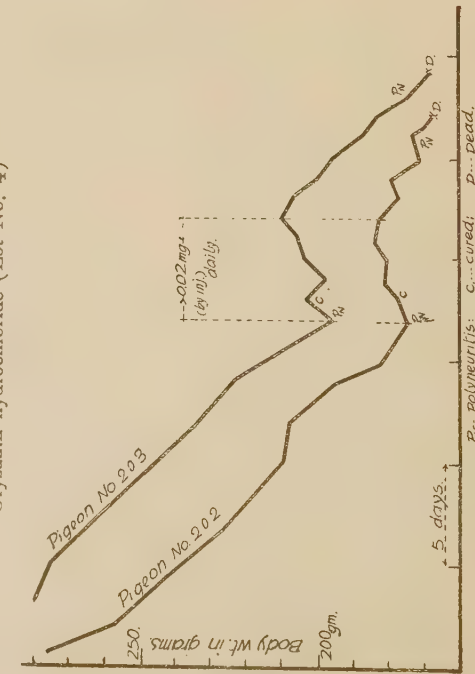


Chart 37—Pigeons on synthetic diet, (Polished rice 92%  
meatprotein 5% McCollum salt 3%) Oryzanin hydrochloride.



Chart 38—Pigeons on synthetic diet, (Polished rice 92%  
meatprotein 5% McCollum salts 3%) Oryzanin hydrochloride.

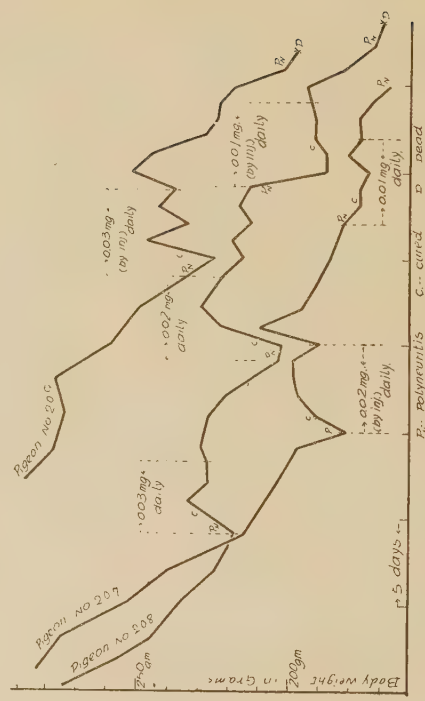


Chart 39—Pigeons on synthetic diet, (Starch 71% meatprotein  
15%, Butter 10% McCollum salts 4%) Oryzanin hydrochloride.  
(Lot No. 3)

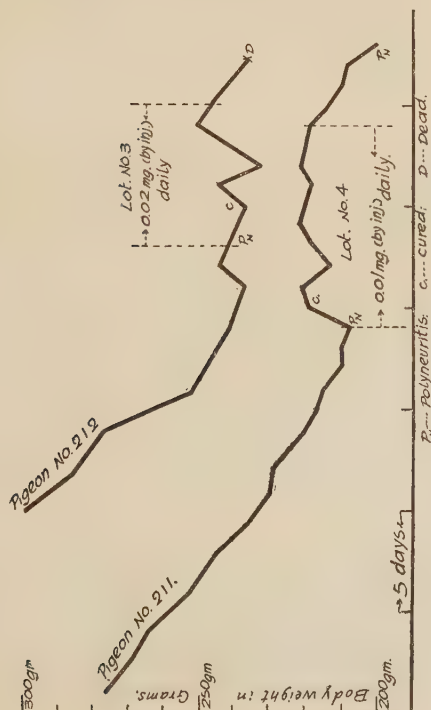


Chart 40—Pigeons on synthetic diet. (Starch 71% meat-protein 15%, Butter 10% McCollum salts 4%). Oryzanin hydrochloride. (Lot No. 3 and 4).



Chart 41—Pigeons on synthetic diet. (Starch 71% meat-protein 15%, Butter 10% McCollum salts 4%). Oryzanin-hydrochloride.

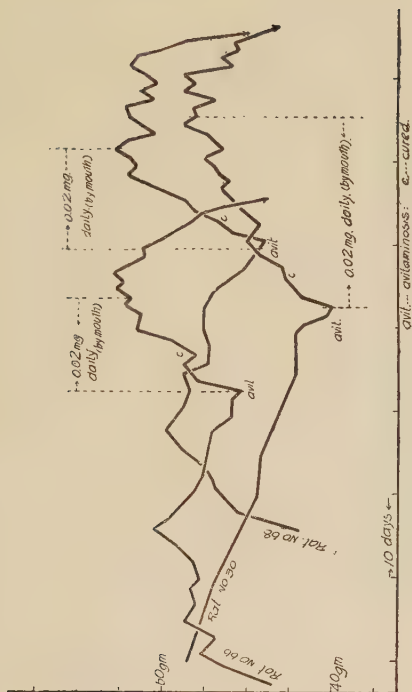


Chart 42—Rats on synthetic diet. (Starch, 70% meat-protein 15%, Butter 10% McCollum salts 5%), Oryzanin hydrochloride.

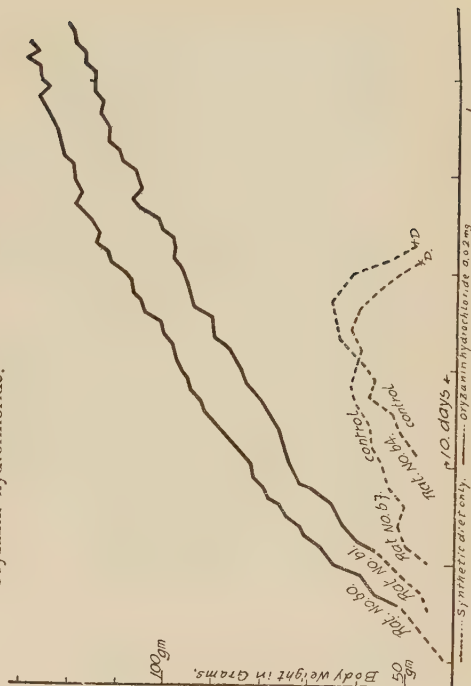


Chart 43—Rats on synthetic diet. (Starch 70% Meat-protein 15%, Butter 10% McCollum salts 5%) Oryzanin hydrochloride.

hours. (Chart. 31~36, Fig. III).

(2) Pigeons when fed previously on the synthetical diet, consisting of 92% polished rice, 5% meat protein or extracted meat<sup>(6)</sup> and 3% McCollum salts mixture<sup>(8)</sup>, developed the symptoms of polyneuritis in 3~4 weeks. By injection of 0.02 mg. daily, the main symptoms were improved within 2~5 hours and cured entirely in 1~2 days (Chart. 37.)

(3) Pigeons fed on the same diet, supplemented with 0.02 mg. daily, remained in perfect health for 50 days against polyneuritis while the symptoms of polyneuritis developed in 10 days when the supplement was withdrawn. (Chart. 38).

(4) Pigeons, when fed previously on the synthetical diet consisting of 71% purified starch<sup>(5)</sup>, 15% extracted meat 10% purified butter<sup>(7)</sup>, and 4% McCollum salts mixture (No. 185), exhibited the typical symptoms of polyneuritis within 4 weeks usually. By injection of 0.01 mg. daily, the main symptoms were improved in 2~3 hours and cured in 1~2 days. In doses of 0.02 mg. or more quantity, it recovered more rapidly and increased body weight markedly to compare it with the pigeons on polished rice. (Chart. 39 & 40, Fig. IV).

(5) Pigeons, fed on the synthetical diet mentioned above, administered with 0.02 mg. of the hydrochloride per os daily, remained in perfect health for 50 days though the bodyweight declined gradually. But the signs of polyneuritis were exhibited in 10 days when the supplement was removed. The supplement of 0.04 mg. daily gave also the same result. (Chart. 41).

[B] *White rats*:

(6) Young rats about 40~50 g. in weight, fed on the synthetical diet consisting of 80% purified starch, 15% extracted meat, 10% purified butter, 5% McCollum's salts mixture and with the addition of 3 drops of codliver oil daily, exhibited the symptoms of neuritis in 4 weeks commonly. By supplementing with 0.02 mg. daily, they were cured quickly and their growth-curve paralleled almost to the normal gaining about 1~2 g. daily. The symptoms developed again on removing the supplement. (Chart. 42, Fig. V & VI).

(7) Young rats, fed on the same diet as above, supplemented daily with 0.02 mg. of the hydrochloride from the beginning of the experiment, grew normally in perfect health for 70 days, gaining the body weight in the rate 1~1.2 g. daily. (Chart. 38).

### Summary.

(1) An antineuritic preparation so-called "Active Oryzanin" was prepared by treating the aqueous extract of rice polishings with Japanese acid-clay and phosphotungstic acid as described above. Its antineuritic activity is found to be in the daily dose of 4 mg. for pigeons as well as for white rats and the



Table II. Isolation of Oryzanin (Antineuritic Vitamin.)

		Grms. for Pigeon.	
Rice-Polishings...1500 kg. (Activity. 4 g...Unit. 375,000...for Pigeon).			
0.2% H <sub>2</sub> SO <sub>4</sub> ...12,000 L.			
Water-Extract.			
Acid clay...105 kg.			
0.5% Ba (OH) <sub>2</sub>			
Alcohol...80%			
Oryzanin Extract...3,750 g. (Activity 12 mg...Unit...312,500...for Pigeon)			
Phosphotungstic acid.			
Active Oryzanin...500 g. (Activity...4 mg. Unit...125,000...for Pigeon).			
H <sub>2</sub> O...15 L...H <sub>2</sub> SO <sub>4</sub> ...75 g...P <sub>H</sub> ...2.6.			
AgNO <sub>3</sub> 500 g. P <sub>H</sub> 2.5.			
-ppt-[Fraction I]-HCl-Phosphotungstic acid-Solid 22 g. Activity...>20 mg.			(1) Adenin .....12.50.....
Ba (OH) <sub>2</sub> ...P <sub>H</sub> 4.5			(2) Adenin Hypoxanthin.....4.50.....
-ppt-[Fraction II]-HCl-Phosphotungstic acid-Solid 48.4 g. Activity...>100 mg.			(3) Adenin Picrate.....0.75.....
Ba (OH) <sub>2</sub> ...P <sub>H</sub> 6.8			(4) Hypoxanthin-Picrate.....0.35.....
-ppt-[Fraction III]-Sol-PtCl <sub>4</sub> -ppt-Insol			(5) Nicotinic Acid.....26.55.....
Phosphotungstic Acid Solid...25 g. Alcohol...0.5 mg. Activity...>5 mg.			(6) Adenin Picrate.....0.65.....
-ppt-[Fraction IV]-HCl-Insol			(7) Nicotinic Acid Picrate.....10.65.....
Phosphotungstic Acid Solid...24.7 g. Alcohol...5 mg. Activity...>5 mg.			(8) Nicotinic Acid Hydrochloride.....2.25.....inactive
Ba (OH) <sub>2</sub> ...P <sub>H</sub> 9.0			Amorph. Resinous Substance .....inactive
-ppt-[Fraction V]-Solid...116 g.-HCl-Alcohol-Sol			(9) Unknown-base hydrochloride .....0.43.....inactive
Phosphotungstic Acid Solid...2.5 mg. Activity...<2.5 mg.			(10) Oryzanin-Hydrochloride .....0.20.....0.01 mg.
-ppt-[Fraction VI]-Alcohol-Sol			(11) Unknown-base Hydrochloride.....0.12.....inactive
Phosphotungstic Acid Solid...5 g. Activity...>10 mg.			Amorph. Resinous Substance .....2.00.....inactive
-ppt-[Fraction VII]-Alcohol-Sol			(12) Nicotinic acid Hydrochloride.....0.09.....inactive
Phosphotungstic Acid Solid...5 g. Activity...>10 mg.			Amorph. Resinous Substance .....4.00.....3 mg.
-ppt-[Fraction VIII]-Alcohol-Sol			(13) Nicotinic acid Hydrochloride.....0.38.....inactive
Phosphotungstic Acid Solid...12 g. Activity...>1 mg.			Amorph. Resinous Substance .....3.25.....inactive
-ppt-[Fraction IX]-Alcohol-Sol			(14) Unknown-base hydrochloride.....0.20.....inactive
Phosphotungstic Acid Solid...24.7 g. Alcohol...5 mg. Activity...>5 mg.			Amorph. Resinous Substance.....6.00.....inactive
-ppt-[Fraction X]-Alcohol-Sol			Amorph. Resinous Substance.....4.10.....inactive
Phosphotungstic Acid Solid...2.5 mg. Activity...<2.5 mg.			(15) Nicotinic Acid Hydrochloride.....0.30.....inactive
-ppt-[Fraction XI]-Alcohol-Sol			Amorph. Resinous Substance.....2.50.....inactive
Phosphotungstic Acid Solid...116 g.-HCl-Alcohol-Sol			(16) Nicotinic Acid Hydrochloride.....17.40.....inactive
-ppt-[Fraction XII]-Alcohol-Sol			Unknown-Base (C <sub>8</sub> H <sub>10</sub> NO <sub>3</sub> HCL)....2.25.....inactive
Phosphotungstic Acid Solid...12 g. Activity...>1 mg.			(17) Cholin Picrate .....5.00.....
-ppt-[Fraction XIII]-Alcohol-Sol			(18) Cholin Picrate .....5.00.....

pigeon curative day-dose to be of the order of 0.74 mg. while it lacks the pellagra protective factor and growth promoting factor.

(2) Starting from "Active Oryzanin" the author isolated the antineuritic vitamin in crystalline state according to the process of Jansen & Donath with minor modification, besides adenin, cholin, hypoxanthin, nicotinic acid, two unknown bases ( $C_3H_6N_2$ )<sub>n</sub> &  $C_8H_{10}NO_3$  etc.

The yield of the antineuritic crystals was very scanty, about 0.85 g. of the purified hydrochloride (1.31 g. of crude crystals) was isolated from 2 kg. of "Active oryzanin" which were prepared from about 6.000 kg. of rice polishings.

These results are summarised in the following schematic table II.

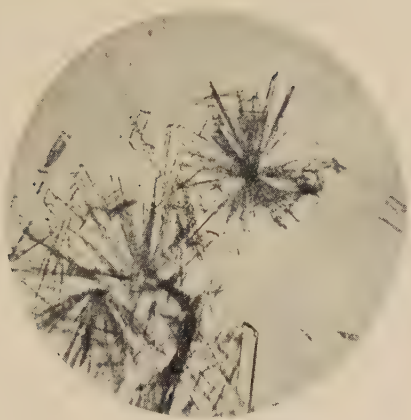
(3) The antineuritic compound isolated by the author, crystallises in colorless long monoclinic plates, melting at 250°C. It gives Pauly's diazo-reaction in quite different coloration to compare with that given by imidazol compound and it contains sulphur in the molecule in a form detectable by sodium nitro-prusside or by lead acetate when it is boiled with alkali or fused with metallic sodium previously. From the analytical results, it was ascertained that the compound should be the hydrochloride of a sulphur compound having the formula  $C_{12}H_{16}N_4SO_2$ . These principal points are not agreed with that reported by Jansen and Donath.

(4) Biological experiment on pigeons and rats showed its antineuritic activity to be in the daily dose of 0.01~0.02 mg. From its activity, the content of antineuritic substance in rice-polishings is presumed to be about 0,0003%.

The further chemical and biological studies on this compound are expected in the next report.

The author express his sincere thanks to Prof. U. Suzuki for his kind advise and encouragement throughout the work. Thanks are due to Mr. T. Yuasa of Sankyo. Co. Ltd. for kind supply of the material. The author is also indebted to Messrs. K. Kamada and T. Yamagishi for their kind assistance, both in chemical and biological experiment. (Apr. 30 th, 1931. Agricultural chemical laboratory, Tokyo Imperial University. Komaba, Tokyo).

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Leitz, III  $\times 4$ .

Fig. I—Oryzanin hydrochloride.



[A] Suffuring from polyneuritis.

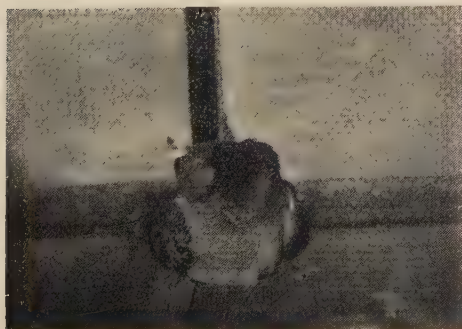


[B] Curing, 2. hrs. after injection with 0.01 mg. Oryzanin hydrochloride.



[C] Cured, 20 hrs. after injection with 0.01 mg. of Oryzanin hydrochloride.

Fig. II—Pigeons [No. 134] on polished rice. (c. f. Chart. 31.)



[A] Suffuring from polyneuritis.



[B] 2 hrs. after injection with 0.02 mg. of Oryzanin hydrochloride.

Fig. III—Pigeons [No. 195] on polished rice. (c. f. Chart. 35.)





[A] Suffering from Polyneuritis (3 weeks.)



[B] Cured, 2 hrs. after injection with 0.03 mg. of Oryzanin hydrochloride.

*Fig. IV—Pigeons (No. 208) on synthetical diet, (c. f. Chart, 39).*



[A] Suffering from Neuritis.



[B] Cured, Supplement, with 0.02 mg. of Oryzanin hydrochloride daily.

*Fig. V—Rat (No. 66) on synthetical diet, (c. f. Chart, 42.)*



[A] Suffering from neuritis.



[B] Cured, Supplement, with 0.02 mg. of Oryzanin hydrochloride daily.

*Fig. VI—Rat (No. 68) on synthetical diet, (c. f. Chart, 42.)*



## The Chemical Research of the Scouring Action of Soap upon Silk.

By

Risaku TSUNOKAYE

(Received March 1, 1932)

The scouring action of soap upon silk has been studied by the author in the Laboratory of Institute of Silk Industry of the Ministry of Commerce and Industry, Yokohama, Japan. The results are as follows:

**I.** The scouring action of soap upon silk can be divided into two parts, first, the degumming action which is to take off the silk gum from raw silk, second, the subsequent effect of soap upon scoured silk. The degumming action of soap upon silk is concluded to the chemical action of alkali, which is developed by the hydrolysis of soap in the water solution, and also to the physical action of colloidal fatty acid soap, the so-called acid soap, which is composed from undecomposed soap and fatty acids, and it has strong adhesive power together with penetrating activity.

One of the characteristic action of soap on the scouring is to scour silk uniformly, that is to say, the silk fibre situated inner part of the fabrics is scoured by the same time with that which is on the out side of the fabrics. This is accomplished by the strong penetrating activity of the colloidal fatty acid soap.

The alkali developed from the hydrolysis of soap, of course, has the power of dissolving sericin, the silk gum, the colloidal fatty acid soap will give negative charge to the sericin or alkali sericin when it is combined with them colloidaly and the sericin or alkali sericin will be removed by their mutual repulsive force from the fibroin.

The degumming action of soap upon silk is most effective when its concentration comes within  $N/50$  and  $N/70$ .

When raw silk is scoured by the soap solution of  $N/50$  using 50 times of its weight, the sericin will be taken off almost entirely when 16% of the soap is decomposed and consumed, but, this does not mean that 84% of soap is supra, because the undecomposed soap will play its part of scouring action by making colloidal fatty acid soap.

**II.** The degumming power of soap upon silk can be determined by measuring the degree of hydrolysis of its water solution.

Among several of the methods of determining the hydrolysis, "Titration Method" represents the best of all its degumming power upon silk.

Among the so-called methods of determining the power of detergency

of the soap such as I "Drop Number Test," II "Foam Number Test," and "Hydrotropie" are examined with the results that the I denotes its degumming power to some extent, but the other two methods have no relation with the power of degumming.

**III.** The scouring powers of several kinds of soaps are compared, the results are as follows:

A. Sodium soap of arachidic, stearic and palmitic acids have good degumming powers, while myristate, laurate, caprate and caprylate diminish their powers of degumming as well as decreasing their molecular carbon numbers.

B. The degumming powers of higher unsaturated fatty acid soaps are good as well as those of the higher saturated fatty acids, when they are fresh and not yet have undergone deterioration.

C. The soaps of hydroxylated fatty acids such as the soaps of di-, tetra-, and hexa-hydroxystearic acids, have generally weak degumming powers and the more OH groups in the molecule of fatty acid the weaker its degumming power.

D. The soaps of tallow, olive oil, safflower oil, arachis oil, soya bean oil, cotton seed oil, chrysalis oil have quite good and the same degumming power when they are fresh, but the degumming power of soaps of cocoanut oil and castor oil are less than those of the soaps mentioned above.

E. The soaps of soya bean oil, cotton seed oil, arachis oil, are liable to go under deterioration in the atmosphere and decrease their power of scouring.

F. The characteristic property of the home made silk scouring soaps is its deterioration in the storage, the consequence of this is that they contain much soaps of unsaturated fatty acids which are easily oxidized by the atmospheric oxygen, and decrease their hydrolysis as well as degumming power.

G. The autoxidation of unsaturated fatty acid or of its soap in the atmosphere differs from the oxidation by means of chemicals such as permanganate. Na-oleate is not easily oxidized in the air. That is because the olive oil soap which is mainly composed of oleic acid is good for the purpose of silk scouring,

**IV.** The substances which affect the nature of the scoured silk are (I) alkali which combines chemically with fibroin, and (II) so-called acid soaps which combines physically with it. The remaining acid soap amounts about 1% of the scoured silk and when the boiled off silk is treated with acid such as acetic or others after scouring, the fatty acid occupy almost all parts of the residual substances, on the contrary, the quantity of soap will increase

when the scoured silk is treated with sodium carbonate.

A. The alkali affects the luster of the scoured silk and when its concentration is high to some extent, the silk will be coloured.

B. As the unsaturated fatty acid undergoes oxidation on the fibre of the scoured silk, the silk is liable to be coloured, but as its soap the silk is not so easily coloured, because the soap does not oxidize so easily as fatty acid on the silk fibre.

C. The scoured silk fibre, as its original nature, will produce to some degree of scroop when it is crushed in hands, but the fatty acid adsorbed on the fibre accompanied with the influence of the acid treatment makes the cause of producing the so-called scroop of the scoured silk.

The scroop produced by the saturated fatty acid is rough compared with that of the unsaturated fatty acids.

V. The silk scoured by the Na-stearate is good in whiteness of its colour, but not in luster. On the contrary, the one from Na-oleate is good in luster but the whiteness is not so good as that of the Na-stearate. The silks scoured by Na-soap of castor oil, tallow etc., which contain mainly saturated fatty acids, are good in whiteness and next come those scoured by olive oil soap and castor oil soap, but the silks scoured by the soaps of chrysalis oil, soya bean oil, cotton seed oil, arachis oil are bad in whiteness. In luster, the silk from olive oil soap is the best, those of the soaps of soya bean oil, cotton seed oil, chrysalis oil, castor oil and arachis oil come next and those of tallow soap and cocoanut oil soap are the worst. The chrysalis oil soap will produce a special bad smell on the scoured silk.

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